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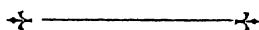
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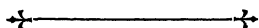
BIOLOGICAL REVIEWS

of the

Cambridge Philosophical Society



Edited
by
H. MUNRO FOX



VOLUME 12

CAMBRIDGE
AT THE UNIVERSITY PRESS
1937

PRINTED IN GREAT BRITAIN

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THE BEGINNING OF THE ~~AGE~~ OF MAMMALS

By GEORGE GAYLORD SIMPSON

(Received 21 January 1936)

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I. ABSTRACT

THE mammals of the Paleocene, first epoch of the Tertiary, the Age of Mammals, are essential for the elucidation of numerous zoological, biological, and geological problems. Among these problems are determination of the affinities of mammals in general, of their ancestral and primitive structures and of the course of their evolution, as well as problems of the origin and nature of adaptations and habits, and more special and, in one sense, practical problems of stratigraphy and some other branches of geology.

The first known Paleocene mammal was described in 1841, but intensive work began with Lemoine's first publication in 1878. Since that time work has continued at ever accelerated pace, by Cope, Osborn, Wortman, Matthew, Granger, Sinclair, Douglass, Gidley, Schlosser, Teilhard, Jepsen, Russell, Patterson, Simpson, and others. A nearly complete sequence of Paleocene mammalian faunas is now known from North America, and more limited but also important faunas are known from Europe, Asia, and South America.

These faunas include multituberculates, marsupials and placental mammals, classified in seventeen orders, the general characters of which in the Paleocene are reviewed. From these mammals it is possible to infer with high probability the ancestral characters of placental mammals in general, the evidence for a primitive

"tritubercular" or trigonal-tuberculosectorial primitive molar type being particularly conclusive and important.

The Cretaceous-Paleocene transition in North America is marked by the disappearance of dinosaurs and the appearance of several orders of mammals apparently as immigrants from some unknown region. The Paleocene sequence on the same continent, which still has two breaks not represented by known faunas, is marked not only by great evolutionary advance but also by progressive enriching of the faunas, chiefly by the appearance of new and generally more progressive mammalian groups as immigrants. The Paleocene-Eocene line is drawn at the culmination of this faunal change. Although in detail the change is by intergradation and gradual transition, from a broader point of view it marks a very radical difference in mammalian faunal type, the Paleocene forms eventually disappearing and the Eocene forms being the forerunners of the later Tertiary and Recent faunas. The same faunal change eventually occurred in South America, but at a much later date, around the end of the Tertiary.

In the Upper Paleocene Asia, Europe, North America, and South America all show considerable local differentiation but give evidence of the derivation of their faunas from a common source. Those of North America and Europe are fairly similar, although not identical, and that of South America is most distinctive, evidence of longer separation from the other continents.

In a general summary of known mammalian faunal history the few known Triassic mammals have no clear significance. The Jurassic mammals of Europe and North America are of distinctive type, with four primitive orders. From two of these developed the multituberculates, marsupials and insectivores of the Upper Cretaceous. Further differentiation of these three, but particularly of the general placental, carnivore-insectivore stock produced the typical Paleocene faunal type. Finally, progressive evolution and diversification of the several Paleocene placental mammal stocks gave rise to the Eocene faunal type which still exists to-day.

II. INTRODUCTION

The most dramatic and in many respects the most puzzling event in the history of life on the earth, as that history is now known, is the change from the Mesozoic, Age of Reptiles, to the Tertiary, Age of Mammals. It is as if the curtain were rung down suddenly on a stage where all the leading roles were taken by reptiles, especially dinosaurs, in great numbers and bewildering variety, and rose again immediately to reveal the same setting but an entirely new cast, a cast in which the dinosaurs do not appear at all, other reptiles are mere supernumeraries, and the leading parts are all played by mammals of sorts barely hinted at in the preceding acts.

This mysterious mortality and this even more mysterious appearance of new forms of life is an unparalleled challenge to the scientific inquirer. On this central mystery he can as yet cast little direct light, but there are many other possibly more important points involved toward the elucidation of which excellent progress is being made. The character of many of the Tertiary mammals, when they do

appear, is now fairly well established, and this is a matter of outstanding general interest and importance. The natural affinities of these early mammals are important from two points of view: looking forward from them, the diversification and dispersion of many of the groups of mammals of the later Tertiary and of to-day may be traced, and looking backward it is possible to determine the probable origins of most of the greater groups and their basic relationships to each other.

The actual anatomical structures of these early mammals, a mass of intricate and boring detail when considered as isolated facts, take on a wider significance the importance of which can hardly be over-emphasized if their bearing on more general problems is considered.

Any conception of the affinities of living mammals, from the duck-billed platypus to man, of their phylogeny, and of the taxonomy which is a means of discussing and, imperfectly, of representing these relationships depends largely on the recognition of those characters that are ancient, primitive, and representative of ancestral stages of various degrees of remoteness, and the distinction of such features from the more recent characters, specializations acquired within the lesser branches of the complex tree of descent. The known ancient fossil mammals have, of course, their own particular adaptations (no generalized animal ever existed), but on the whole they are decidedly primitive and some of them are also actually or structurally ancestral. They provide the most direct and reliable approach to many problems with which comparative anatomists and biologists in general struggle in vain when only surviving mammals are considered.

Intimately related to such problems and forming the ultimate basis for their solution are more specific questions of comparative anatomy. On some of these, such as the differentiation of mammalian tooth patterns, the early mammals give data that far outweigh in value all else that is known. In many others, such as the development of the mammalian brain, they add priceless items to the available data.

In the field that has come to be called inappropriately paleobiology, which is in fact a combination of paleophysiology and paleoecology, the early mammals also make important contributions. Much can be determined and more inferred with great probability as to the habits of these ancient forms and their ecological relationships to each other. This has a strong bearing not only on such obviously related studies as those of the development of mammalian adaptations, habits, and instincts, but even on such branches of science, at first sight apparently so remote, as, for instance, psychoanalysis or orthopedics.

There are many other essential contributions made to biological sciences, but the importance of the early Tertiary mammals does not stop there. Knowledge of them is essential in dealing with many of the primarily physical problems of geology and geography, notably in the study of the development of the continents and the connections between them and in checking the recent theory of continental drift. Then there are thick series of rocks on several continents the stratigraphy, correlation, and age of which can only be studied in the light of these early mammalian faunas. This even brings an immediate commercial or so-called practical value to a study that otherwise might typify a pure or unapplied science, for in several areas

the faunas in question are of material assistance in the search for petroleum and other mineral resources.

Enough has been said to show that the studies here reviewed are not isolated but occupy a definite and in some respects a very crucial position in the anatomy of science, and to show in a general way what that position is. Intensive work in this field may be said to date from 1878, when Lemoine began describing the Cernaysian fauna of France, and it has been continued at a constantly accelerated pace ever since then. Study is now very active and some of the most important work has been done in the last ten years. Indeed much of it has not yet been published. The rapidity of discovery, the special, almost esoteric, nature of the detailed data, and limited dissemination of knowledge of these facts have made it difficult for the more numerous workers in related fields to appreciate that here is information vital for understanding their own problems, or to find and interpret the information even if they are aware of its existence. These circumstances are the justification for the present essay, and filling these needs, as far as this can be done in a brief space, is its aim.

It is first necessary to limit and define the field to be covered. The primary concern here is with the Paleocene mammals. The Paleocene Epoch is the oldest of five divisions of the Tertiary Period, popularly characterized as the Age of Mammals and covering the time from the end of the Cretaceous, roughly 60,000,000 years ago, until the beginning of the Pleistocene, about 1,500,000 years ago. The duration of the Paleocene is not known with any accuracy, but from various considerations it must have covered about one-fifth of the Tertiary, that is, in the neighbourhood of 10,000,000–15,000,000 years.

There is as yet no completely general agreement as to the stratigraphic and faunal limits of the Paleocene. Some geologists still do not recognize it as an entity, and some of those who do so recognize it refuse to use that name and prefer to subordinate it as a part of the preceding or of the following epoch; significantly, those who follow this course are also those whose work betrays the least familiarity with many of the facts here reviewed. There is also some disagreement as to the exact place at which the boundaries, especially the boundary against the following epoch, should be drawn. It is not proposed to enter into any detailed consideration of such arguments here. They are purely taxonomic and largely verbal, and they have no bearing on the general succession and nature of the faunas, which are not open to disagreement. For present purposes, it is assumed that the Paleocene is a separate and major division of the Tertiary, comparable in duration, scope, and distinctiveness with any of the four universally recognized later epochs. It is further assumed, in agreement with the consensus of students of Paleocene mammals, that the Paleocene in North America, where it is much better known than anywhere else, includes the Puerco Formation below and the Clark Fork Formation above, and hence includes all formations and faunas equivalent to or intermediate between these two in age.

In preparing this review I have drawn not only on the literature and my own published papers, but also on personal knowledge of much unpublished material,

principally in the American Museum of Natural History, New York, and the United States National Museum, Washington, and on personal communications of unpublished observations from the late Prof. W. D. Matthew, from Dr G. L. Jepsen (Princeton University), and from Father T. E. Reynolds (St Louis University). Dr Matthew's final revision of the Puerco and Torrejon faunas, which he left in nearly completed form and which will be published as soon as possible, has also been carefully read and has been of the greatest assistance in attempting to summarize these large faunas, originally described in about seventy different papers by a dozen authors.

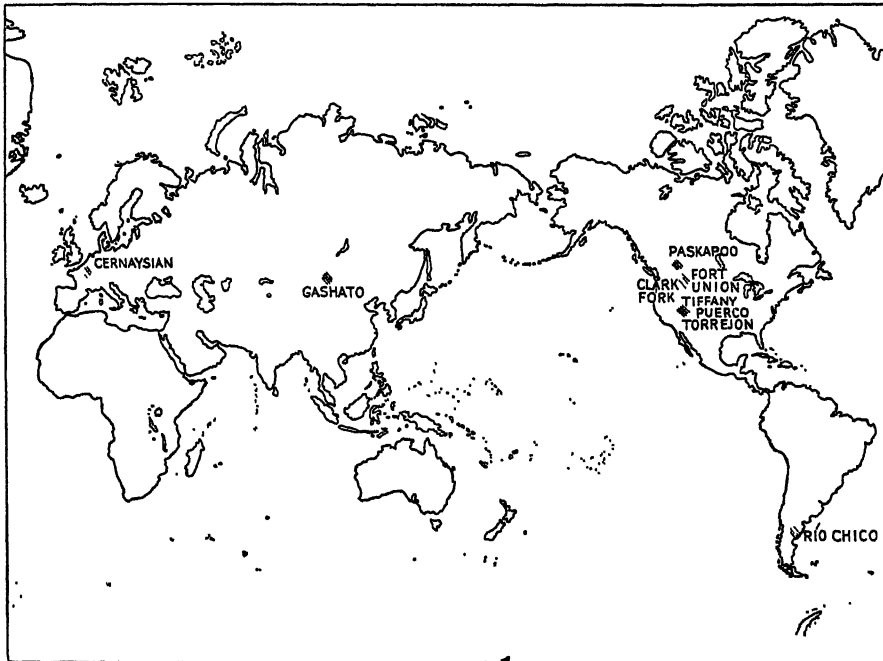


Fig. 1. Geographic distribution of the principal known mammal-bearing Paleocene formations of the world.

The references given at the end of this paper are not intended as a complete bibliography or documentation of every statement made, but should make available to the non-specialist the most important, most recent, and most inclusive original research on the subjects treated. Older or more detailed publications can all be readily found from the bibliographies of those here cited.

III. EXPLORATION OF THE PALEOCENE

A rapid historical review of the discovery of Paleocene mammals serves to give an example of the accretive process by which knowledge is attained and also to mention the known fossil deposits and their geographic and geologic positions and to show the amount and nature of progress made in their study.

(1) *Europe*

Cernaysian. The first Paleocene mammal ever to be discovered was a skull of *Arctocyon primaevus*, from La Fère, France, named and described by de Blainville in 1841. This remained an isolated occurrence for over thirty years. In 1873 Aumonier and Eck made a detailed study of the Mont de Berru, a hill at the village of Cernay, near Reims, and in the same year Victor Lemoine began collecting fossil mammals in the stratum which they named the "conglomerat de Cernay". This deposit is intercalated between the Cretaceous chalk and the unquestionably Eocene Sparnacian, and its mammals were older than any previously known from the Tertiary with the single exception of the contemporaneous *Arctocyon* of de Blainville. They are now referred to the Upper Paleocene. Some of Lemoine's first specimens were sent to Gervais for study, but Lemoine soon undertook to classify them himself and published a series of twenty-six papers on them between 1878 and 1896, the year before his death.¹

A few fragments of the same age and of no great importance have been found elsewhere, but for all practical purposes the only known European Paleocene mammalian fauna is that of Cernay. The Cernaysian proper is a local stratum referable to the Thanetian, a more general term for the Upper Paleocene of the Paris-London Basin, but the Thanetian is elsewhere almost barren of mammals. The fauna has been carefully revised by Teilhard de Chardin (1916-21), and I have elsewhere reviewed his work and added a few comments (Simpson, 1929 b).

(2) *North America*

Puerco and Torrejon. The second region in which Paleocene mammals were found, and by far the most important yet known, was the San Juan Basin, in north-western New Mexico, south-western United States. The Puerco series was discovered and named by E. D. Cope when he visited this area in 1874, as paleontologist for the Hayden Survey, but he then found no fossils in it except petrified wood. In 1880 Cope engaged David Baldwin, a local collector who had previously worked for Cope's rival, O. C. Marsh, to examine these beds, and Baldwin was phenomenally successful. He worked these intermittently in 1880-8 and sent Cope a large collection, described by the latter in a series of thirty-five papers from March 25, 1881 to 1888 (and mentioned or discussed in numerous others), the last paper a summary of the whole fauna as known to him (see Cope, 1888). In addition to this intensive description, the Puerco fauna entered into most of Cope's more general and theoretical papers after 1881 and indeed colored his whole viewpoint, as, consciously or unconsciously, it has that of every competent mammalogist since then.

When the Department of Vertebrate Paleontology of the American Museum of Natural History was established under Henry Fairfield Osborn, one of its first activities was the resumption of exploration of the Puerco by parties led by J. L. Wortman in 1892-6. In 1895 Cope's collection was purchased by the American

¹ For citations of these papers and others descriptive of the Cernaysian fauna see Teilhard (1916-21), references at end of this paper.

Museum. Matthew noted that in the nineties our collection included some 1500 specimens from the Puerco and Torrejon, and the number has been about doubled since then. These collections were described by Osborn & Earle (1895), Wortman (1896), and Matthew (1897). Among the many important discoveries made was the fact that Cope's Puerco included two totally distinct faunas, to the older of which the name Puerco was restricted while the younger was called the Torrejon.

Large Puerco and Torrejon collections were again made by American Museum parties under Walter Granger in 1913 and 1916, and some Puerco material was added by me in 1929. Several smaller collections have been made by others. A very brief note was published by Matthew & Granger in 1921, and there are a few other notes of details or casual references, but the great bulk of this more recent material, including much more perfect material than any that has been published, has never been illustrated or described. This great desideratum was to be supplied by a memoir nearly completed by W. D. Matthew before his death in 1930, still in manuscript but to be published as soon as funds permit.

The stratigraphy of the beds was definitely established by Sinclair & Granger (1914). Despite the marked faunal differences, the Puerco and Torrejon appear to be continuous in the field and cannot be certainly distinguished except by their fossils. Like the Cernaysian, they are intercalated between undoubted Cretaceous strata (Ojo Alamo) and the Lower Eocene (Largo and Almagre).

Tiffany. In 1916 Granger made a collection of fossil mammals from beds to which he applied the name Tiffany, in south-western Colorado, near to and north of the classic Puerco and Torrejon exposures. A few fragments had previously been found there by J. W. Gidley. The formation was recognized as being younger than the Torrejon, but still older than the undoubted Lower Eocene "Wasatch" = Sand Coulee and Gray Bull of Wyoming or Largo and Almagre of New Mexico (approximately equivalent to the Sparnacian beds of Europe). The occurrence and stratigraphy were discussed by Granger (1917), and some of its fossils have been briefly described by Matthew & Granger (1921). Full descriptions and revision have recently been completed (Simpson 1935 *b, c, d*).

Fort Union. The very widespread coal-bearing Fort Union group of the north-western American Plains, Montana, Wyoming, and the Dakotas was named and described by Meek and Hayden in 1862, and ever since that date has been the subject of intensive study by scores of American geologists. Despite this long examination, it was not until 1901 that mammals were found in these beds by Earl Douglass, working for Princeton University east of the Crazy Mountains in Central Montana. In 1902 and 1903 Princeton parties under Farr also worked in this area, but all these early collections were very fragmentary and revealed little beyond the bare fact of the presence of mammals similar to those of the Torrejon. A local collector, A. C. Silberling, accompanied the Princeton parties and has collected mammals in the Fort Union as opportunity presented ever since their time. In 1908-11 his work was supported by the United States Geological Survey and the United States National Museum, and in those years he made a splendid collection now the property of the latter institution. J. W. Gidley published a few

preliminary notes on part of this material (see Gidley, 1923), but most of it has never been studied or published.¹ In 1930 Silberling also collected for the American Museum of Natural History, and more intensive work for that institution by Silberling, me, and others in 1935 resulted in far the largest collection yet made in the Fort Union.

Most of the mammals of the Fort Union in the Crazy Mountain area are equivalent to the Torrejon in age, but the facies is very different and many of these Fort Union fossils are unlike those known from New Mexico, notably a series of primates, the most ancient known.

A few fragments of mammals, mostly indicating equivalence to the Torrejon, have been found at scattered localities elsewhere in the Fort Union, but the only important discoveries so far made outside the Crazy Mountain Field are at Bear Creek, in southern Montana, and in Park County, northern Wyoming.

In 1926 J. F. Lobdell² found mammals immediately above a coal seam in the Fort Union at Bear Creek, Montana. Later small collections were made by Barnum Brown and by Miss Husband (now Mrs Nichols) for the American Museum and by J. L. Kay for the Carnegie Museum, and the fauna has been described by me (Simpson, 1928 *a*, 1929 *a*). Although in the Fort Union Group, the fauna is later than the principal fauna of the Crazy Mountain area, and seems to be equivalent in age to the Tiffany, although of different facies.

It had long been suspected that the great thickness of the Fort Union included equivalents not only of the Torrejon, or even of the Torrejon and Tiffany, but also of the Puerco. This, however, had not been established by the discovery of fossil mammals, nor had the whole American Paleocene sequence ever been found in one continuous series of strata until the work of Princeton University parties under G. L. Jepsen in 1927-9. In Park County, Wyoming, not far south-east of Bear Creek, Montana, four distinct superposed faunas were found in the Fort Union and associated strata and the rather small but characteristic faunas from each showed them to be equivalents, from oldest to youngest, of the Puerco, Torrejon, Tiffany, and Clark Fork (for which see below). All the Paleocene mammal horizons yet distinguished were thus established beyond question in their true order. Stratigraphy and faunas have been described by Jepsen (1930), who is continuing work in the same area and has since found other valuable material not yet published.

Clark Fork. The Clark Fork beds of northern Wyoming (called Ralston in the first publication and changed when that name was found to be preoccupied) were first distinguished by Sinclair and Granger in 1911. Without knowledge of their fauna, they had previously been included in the typical Lower Eocene, the so-called Wasatch, but their mammals proved them to be distinctly earlier and definitely separable (Sinclair & Granger, 1912; Granger, 1914). They were recognized as

¹ Since Dr Gidley's death the collection has been placed in my hands and a monograph on it is in progress and will probably be completed in 1936. Silberling and I also made a map and stratigraphic studies in 1932 for the United States National Museum.

² This discovery is generally credited to J. C. F. Siegfriedt. Dr Siegfriedt deserves much praise for bringing the occurrence to scientific attention, but the actual discovery and much of the collecting were by Lobdell.

being of a more ancient and essentially Paleocene faunal type intermediate between the Torrejon and the true Lower Eocene. The subsequent discovery of the Tiffany, occupying a similar position in the sequence, suggested approximate equivalence of the Clark Fork and Tiffany. There was, however, some suspicion that the Clark Fork was slightly later, and this was proven by Jepsen's discovery of equivalents of each in superposition, already mentioned. The fauna was mostly included in Matthew and Granger's revision of the Lower Eocene faunas (Granger, 1915; Matthew, 1915 *b, c, d*, 1918), was added to by Jepsen (1930) and is summed up and annotated in a paper by me (Simpson, 1936).

Paskapoo. Like the Fort Union, which it resembles and replaces in the more northern plains, in Alberta, Canada, the Paskapoo was known and named (by Tyrrell in 1886), long before mammals were discovered in it. This discovery was made in 1910 by an American Museum party under Barnum Brown, and was announced by W. D. Matthew in 1914. Unfortunately the specimens were at first confused with some from another formation, obscuring their real age, but this error was corrected. The very scanty known fauna was summed up by me in 1927, and Russell has since made several small but interesting additions (1929, 1932). All the fossils so far known belong in the Upper Paleocene, equivalent to the Tiffany and Clark Fork, but as in the case of the Fort Union it is entirely possible that the Paskapoo also included older Paleocene horizons that have not yet yielded mammals.

Ruby. The Ruby Formation was also known for many years before vertebrate fossils were found in it. This discovery was made by a local collector, E. B. Faber, in 1929, and has since been supplemented by Field Museum parties under Bryan Patterson. The fossils are still very few in number, but as described by Patterson (1933, 1934) they show that the lower part of the Ruby is of Upper Paleocene, possibly Clark Fork, age and the upper part Lower Eocene.¹

(3) *Asia*

Gashato. In 1923 and 1925 the Central Asiatic Expedition of the American Museum of Natural History, under R. C. Andrews, Walter Granger, paleontologist, discovered and collected from a Mongolian formation which is thus far the only mammal-bearing Paleocene deposit known in Asia. It was named and the first fossils described by Matthew & Granger (1925), and additions were made and the whole fauna revised and discussed by Matthew, Granger & Simpson (1928, 1929). It seems definitely to enter into the Paleocene, although probably near the end of that epoch, and its fauna of eleven genera and twelve species is one of the most peculiar and interesting yet found in the Paleocene. Subsequent efforts to add to knowledge of this important horizon have all been blocked by the Asiatic powers.

(4) *South America*

Río Chico. The correlation of the earlier South American Tertiary formations in Patagonia is peculiarly difficult, largely because of the very limited resemblance of their fossils to those of any other part of the world. Consequently there is as yet

¹ Since this paper was in type, Patterson has applied the name *Plateau Valley* to the Paleocene part of the Ruby.

no general agreement as to what formations, if any, represent the Paleocene there. Until recently the Casamayor Formation, "Notostylopense" of Ameghino, was the oldest recognized mammal-bearing formation.¹ Ameghino placed its fauna in the Cenomanian, but this is surely incorrect and has not been supported by any subsequent worker. Some students (*e.g.* Ihering, Kraglievics, Rusconi) place it as Paleocene or as partly Paleocene and partly Lower Eocene. Others (*e.g.* Matthew) place it in the Upper Eocene. The consensus, at least outside the Argentine, now is that it is Eocene, in whatever part of that epoch it may belong. As a result of a more thorough study than has otherwise been made, at least since Ameghino, I am convinced that it is Eocene, probably Lower but possibly Middle Eocene.

In 1930 A. Piatnitzky, a geologist working for the Argentine government, found mammals below the Casamayor, in strata then universally considered as of Cretaceous age. In 1931 and 1934 the Scarritt Expeditions of the American Museum made an intensive study of these beds and collected from them a varied but fragmentary fauna. Lesser collections have been made by E. Feruglio, J. Brandmayr, A. Bordas and possibly others. The fauna and stratigraphy, described by me (Simpson, 1935 *a, e*) clearly show that the formation belongs in the Tertiary. It may be Lower Eocene, but there is slightly greater possibility that it is Upper Paleocene, or it may cover a considerable period and extend even lower into the Paleocene. In any event the formation, which I have named the Río Chico, includes the only mammal-bearing beds yet known in South America that are probably referable to the Paleocene.

IV. SUMMARY OF KNOWN PALEOCENE FORMATIONS AND FAUNAS

There are numerous formations throughout the world that are of known or suspected Paleocene age but in which no mammals have yet been found. These are not listed or discussed here. The following are the principal mammal-bearing formations the discovery and general nature of which have now been mentioned and the faunas of which are discussed in the later pages of this review:

Europe: The Cernaysian, a member of the Thanetian, in the vicinity of Reims.

Asia: The Gashato, at Shabarakh Usu, in Outer Mongolia.

South America: The Río Chico, in central Patagonia.

North America: The accompanying table (Fig. 2) gives the names and correlation of the principal mammal-bearing Paleocene formations as now understood. In addition to those shown, the Ruby Formation of western Colorado is Paleocene in its lower part and has yielded at least two genera of Paleocene mammals, and a few have been found at other scattered localities, especially in the Fort Union. Future usage will perhaps extend the very ambiguous term "Fort Union" to include the whole Paleocene and will provide more definite names (than, for instance, Fort Union "Tiffany" level, etc.) for divisions corresponding with faunal changes. The name "Fort Union" and those of its stratigraphic subdivisions such as "Lebo" are now applied differently by every different worker and are defined on

¹ With the exception of several erroneous or extremely dubious reports of mammals in true Mesozoic strata.

inconclusive physical criteria not corresponding with any logical faunal or temporal divisions. The double lines in the chart represent faunas that are believed to be missing from the known sequence, but if so will probably be found at a known stratigraphic horizon in some area.

DIVISIONS OF THE PALEOCENE	San Juan Basin, Colorado and New Mexico.	Northern Wyoming and Southern Montana.	Crazy Mountain Field, Montana.	Alberta, Canada.
UPPER	(Not yet identified)	CLARK FORK	?	PASKAPOO
	TIFFANY	Tif- fany	BEAR CREEK FORT (Known faunas Torrejon and somewhat later in age).	-----?
MIDDLE	TORREJON	FORT UNION	UNION	(Faunas not yet identified, perhaps included in Paskapoo Strata).
LOWER	PUERCO	"Puerco"	TULLOCK (Mammals unknown, age doubtful).	

Fig. 2. Tentative correlation of the principal known mammal-bearing Paleocene formations of North America.

V. CONSPECTUS OF PALEOCENE MAMMALS

Multituberculata. The multituberculates constitute a peculiar side branch of the mammals that arose in the Triassic and henceforth evolved independently of all other types. Their relationship to the typical Tertiary and recent mammals is thus exceedingly distant. Seduced by the great antiquity of the group and certain hypothetical considerations, a few students have maintained that the multituberculates really do represent the ancestry of some or all later mammals, but all workers who have a competent first-hand knowledge of the specimens agree that this is impossible. In all known Mesozoic mammalian faunas multituberculates are an abundant or the dominant element. In the Paleocene they are still common, although much overshadowed in importance by other, more progressive orders, and a few stragglers survived into the Eocene but there soon became extinct. In the Paleocene twelve genera of multituberculates are now known, but these all fall into three adaptive types. In one, least specialized since it is structurally nearest to the remote Jurassic ancestry of all, the slender, enlarged incisors are more fully enamel-covered and are a cropping, not gnawing, apparatus. There is one very large lower premolar, compressed laterally into a strong, serrated shearing blade. The molars, as in all multituberculates, have parallel longitudinal rows of cusps, and in this

group of which *Ptilodus* is the best known genus, the cusps are rather delicate and have a very complex basically selenodont form. In another closely related group typified by *Eucosmodon*, the principal modification is that the incisors are laterally compressed and have a limited band of enamel, analogous to the condition in rodents although, unlike rodents, the multituberculate incisors form closed roots. The premolars are similar in form to those of the *Ptilodus* group. In the third group, so distinctive that it is generally classed as a separate family based on the genus *Taeniolabis*, the incisors also have an enamel band but are larger and heavier, the

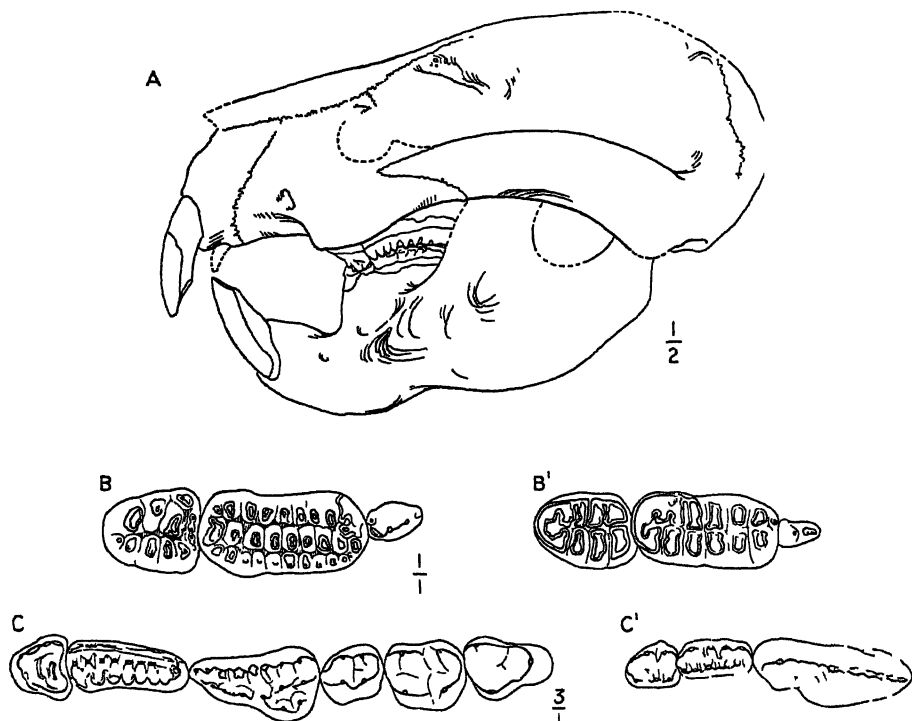


Fig. 3. Paleocene multituberculata. A, *Taeniolabis taoensis* (Cope), left side of skull and jaws. B, same species, crown view of right upper cheek teeth. B', same species, crown view of left lower cheek teeth. C, *Ptilodus mediaevus* Cope, crown view of left upper cheek teeth. C', same species, crown view of right lower cheek teeth. All based on Granger & Simpson, redrawn and somewhat modified. Enlargements as indicated.

premolars are much reduced and have lost the shearing function, and the molars are relatively larger and more stoutly constructed. *Taeniolabis taoensis*, the largest known multituberculate, has a skull about six inches long, and the other species range down to very diminutive shrew-like forms.

Marsupialia. North American Paleocene marsupials, and a doubtful European form, are opossums so close to the modern forms that they have relatively little morphological interest except to demonstrate the antiquity and conservatism of the group. In South America the borhyaenids, predaceous marsupials, appear in the Paleocene in very primitive form, of a synthetic didelphid-dasyurid type. The most

extraordinary Paleocene marsupials belong to the South American family typified by *Polydolops*. In their enlarged incisors, serrate shearing teeth, and multicuspid (but not technically multituberculate) molars they parallel the multituberculates so closely that they were long confused with that group, to which, nevertheless, they certainly do not belong. They were still common in the Eocene, but soon died out there, as far as the record shows, concomitant with and probably because of

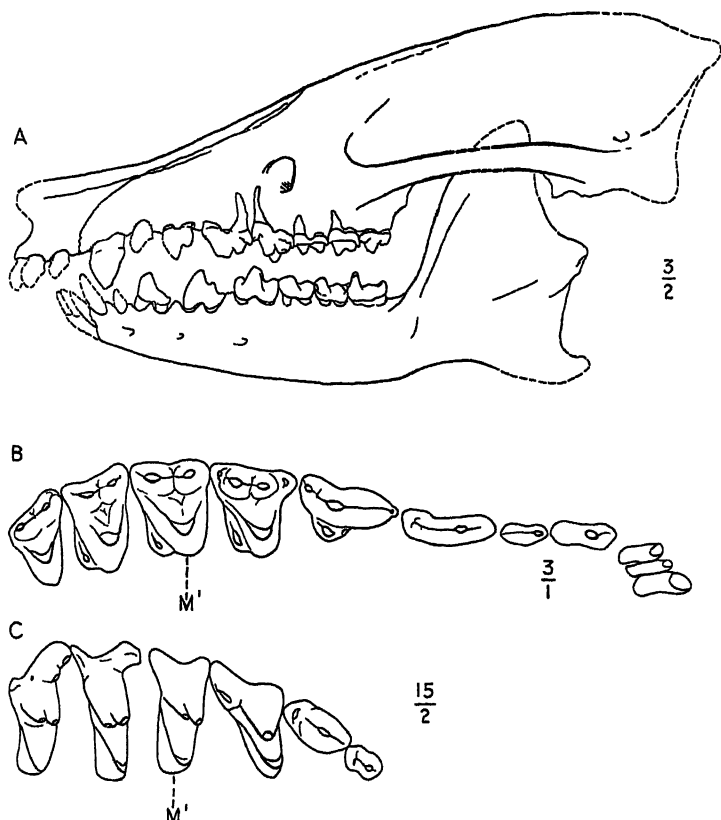


Fig. 4. Paleocene and Lower Eocene Insectivora. A, *Diacodon bicuspis* (Cope), a Lower Eocene member of the Leptictidae, a family also well represented and closely similar in the Paleocene, left side view of skull and jaws. B, *Prodiacodon puericensis* (Matthew), a Middle Paleocene leptictid, crown view of right upper dentition. C, *Palaeoryctes puericensis* Matthew, a Middle Paleocene zalambdodont, crown view of right upper cheek teeth. A and B based on Matthew, C on Simpson, all redrawn and modified. Enlargements as indicated.

the assumption of a similarly rodent-like habitus by certain notoungulates and shortly before (perhaps also concomitant with, were the record complete) the appearance there of true rodents.

Insectivora, etc. Insectivores in general were small forms not readily preserved and difficult to find, but about twenty-five genera have been identified in the Paleocene. The majority of them are leptictids, a group related but not ancestral to the hedgehogs, known from several American formations and from Cernay. Among

the most interesting of known Paleocene insectivores¹ is *Palaeoryctes*, from the Torrejon, a very primitive genus allied to the so-called zalambdodonts, an assemblage of several groups now living on Madagascar, Africa, and the West Indies. Its greatest importance is that it shows the mode of origin of the peculiar trigonal molars of these modern insectivores, which have figured very largely in theories of evolution of the mammalian dentition.

In addition to these undoubted insectivores, there are about a dozen other genera in the American Paleocene, referred to four or five families, that are more tentatively placed in the Insectivores. They are of interest chiefly to the specialist, as the affinities of most of them are dubious, none seems to cast much light on the ancestry of later forms, and they do not make up an important part of the faunas.

The tillodonts, represented by *Esthonyx* in the Clark Fork, may be passed over as an essentially Eocene group appearing at the end of the Paleocene. The plagio-menids are represented by *Planetetherium* at Bear Creek but better known from *Plagiomene* in the Lower Eocene. They somewhat resemble the living *Galeopithecus*, but there is no certainty that this indicates a real relationship.

Zanycteris of the Tiffany is another very puzzling form which merits only passing mention. Its molars resemble those of very specialized recent phyllostomatid bats, more than they do any other known forms, but in some other respects it is unlike the Chiroptera and its affinities are very dubious.

Primates. The very large Puerco and Torrejon collections contain no trace of true primates, but the influence of facies on knowledge of fossil faunas is well shown by the fact that primates are abundant in the Fort Union at levels of Torrejon age (see especially Gidley, 1923). The six genera there found are all closely similar and seem to belong to a single ancestral stock, yet they show incipient divergence into at least three groups that in later beds become very distinctive. *Promothodectes* is nearly or directly ancestral to *Plesiadapis*, a genus common both in the American Upper Paleocene and at Cernay, and through it to *Platychoerops* of the European Lower Eocene, after which this line apparently became extinct. The most obvious character of this group, but one that also appears in many others, is the great enlargement of one pair of upper and one of lower incisors. Some students have held that the plesiadapids are not primates at all, but insectivores (*e.g.* Matthew, who, however, abandoned this view in his last years, and Gregory), and others (*e.g.* Stehlin, Abel) have supposed them to be ancestral to *Daubentonia* ("*Chiromys*"). Recent study of unusually complete specimens from the Tiffany (Simpson, 1935 c) shows beyond much question that they are true primates, a side branch of the lemuroids, with no recognizable special affinity to *Daubentonia*.

Elphidotarsius represents the beginning of another side line, continued in *Carpodaptus* and *Carpolestes* of the American Upper Paleocene, noteworthy for the development of serrated shearing teeth, paralleling the multituberculates and the polydolopids (for a full discussion of these remarkable instances of convergence see Simpson, 1933). This rapidly specialized phylum is not known in the Eocene and apparently had no later descendants.

¹ Along with a similar but even more primitive genus not yet published (Reynolds).

The other genera of Torrejon age, *Paromomys*, *Palaechthon*, *Plesiolestes*, and one other, are more generalized types showing, like most of the Eocene mooted tarsioids, some incisor specialization but primitive in general structure. They are clearly primates and are very important in revealing such an ancient and primitive

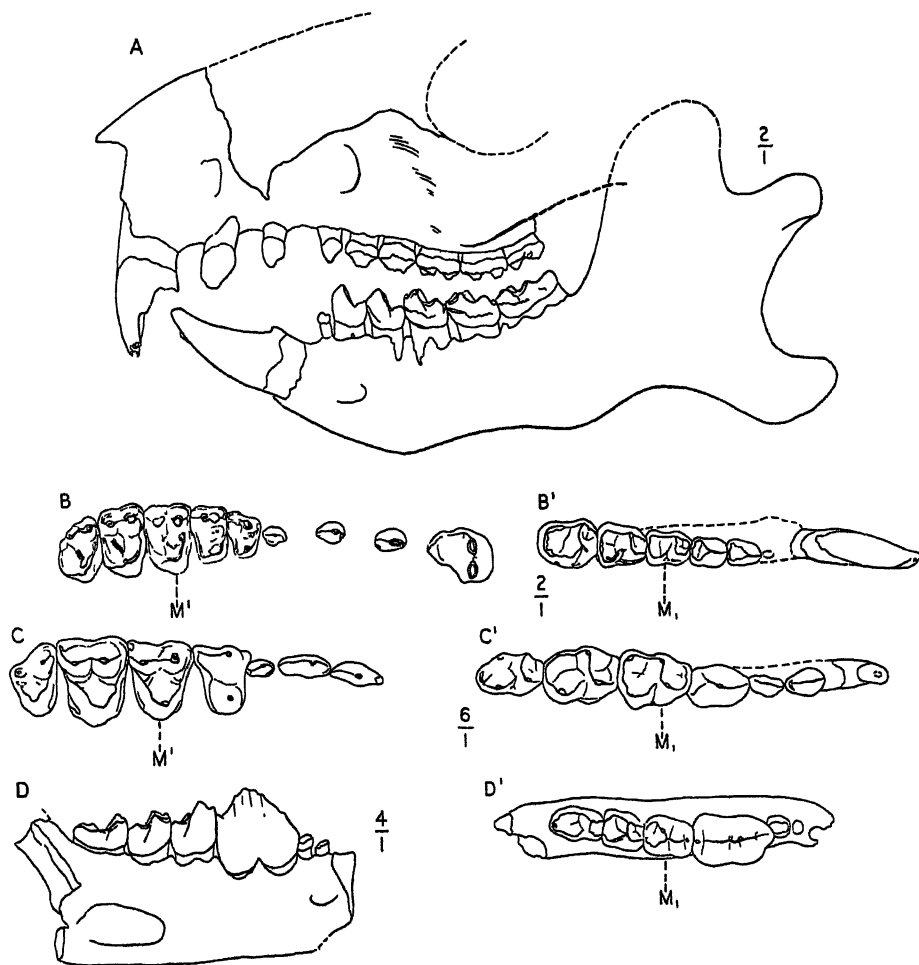


Fig. 5. Paleocene Primates. A, *Plesiadapis gidleyi* (Matthew), left side view of jaw and part of skull. B, same species, crown view of right upper dentition. B', same species, crown view of right lower dentition (the heel of the last molar considerably foreshortened). C, *Navajovius kohlhaasae* Matthew & Granger, crown view of right upper cheek teeth. C', same species, crown view of right lower dentition. D, *Carpodactes aulacodon* Matthew & Granger, external view of part of right lower jaw. D, same, crown view. All based on Simpson, redrawn and modified. Enlargements as indicated.

type of dentition in this group. It is impossible to ascertain surely whether they are nearer to the lemuroids or the tarsioids. Probably these two groups were very little or not at all differentiated at that time and these Paleocene genera are in fact related to both. In the Upper Paleocene this general type of primates is so far represented only by *Navajovius* of the Tiffany.

A more specialized group, characterized by rodent-like gnawing incisors, first appeared in *Labidolemur* of the American Upper Paleocene, is represented by progressively more advanced forms throughout the American and European Eocene, and is last seen in *Sinclairiella* of the American Lower Oligocene. This phylum has generally been confused with the Plesiadapidae, but Jepsen (1934) has recently shown it to be quite distinct.

The only other known Paleocene primate is *Phenacolemur* of the American Upper Paleocene. The genus also occurs in the Lower Eocene, but its closer affinities are unknown.

Taeniodonta. The taeniodonts are a very peculiar group of early ungulates some members of which paralleled the ground sloths to such an extent that Wortman (1896) considered them actually ancestral to the latter, a view now untenable. Two distinct phyla appear in the American Paleocene. In one, *Onychodectes*, Puerco, and *Conoryctes*, Torrejon, the size is moderate and the teeth remain low-crowned and primitive. In the other, *Wortmania*, Puerco, *Psittacotherium*, Torrejon, *Calamodon*, Lower Eocene, and *Stylinodon*, Middle Eocene, the later forms are of bear-like size and the teeth become long and rootless, with limited enamel bands on the sides. They do not seem to be closely related to any other mammals, except through a probable Cretaceous proto-insectivore common ancestry.

Edentata. Very primitive edentates appear in the latest Paleocene, *Palaeanodon* in the Clark Fork. This group is much better known from the Eocene (see Simpson, 1931) where very well preserved skeletons show that it is a conservative line derived from the ancestry of the Xenarthra. True Xenarthrans appear in the South American Río Chico, but are represented only by isolated armadillo scutes. Good skeletal material (unpublished) from the Eocene, Casamayor, shows that the Xenarthra and its subdivision the Loricata had there already acquired their basic characters, but still retained primitive features pointing back to a more *Palaeanodon*-like ancestry.

Rodentia. The discovery of a *Paramys* at Bear Creek (Jepsen, personal communication) carries true rodents back into the Upper Paleocene, without altering the fact that they appear there only as the forerunners of an Eocene invasion. *Eurymylus* in the Mongolian Gashato is probably, but not surely, a rodent, but like most of the Gashato fauna it is very peculiar, with some remarkably primitive characters and yet aberrant in a way that probably removes it from any main line of evolution toward the known later mammals.

Carnivora. Placental carnivores are abundant and varied in the American Paleocene and present in the Cernaysian and Gashato, but did not reach South America until the Pliocene. All the Paleocene carnivores are creodonts, members of an archaic carnivore radiation from one branch of which (the Miacidae or close allies) arose a second radiation leading to the later Tertiary and recent modernized carnivores. The great majority of known Paleocene carnivores, twenty-two valid genera (one still unpublished), belong to the most primitive of creodont groups, the Procreodi or Arctocyonoidea (families Arctocyonidae and Triisodontidae), in which

no carnassial teeth, such as occur in the more specialized creodonts and all fissipeds, had been developed. They are all basically alike, with the dentition complete or

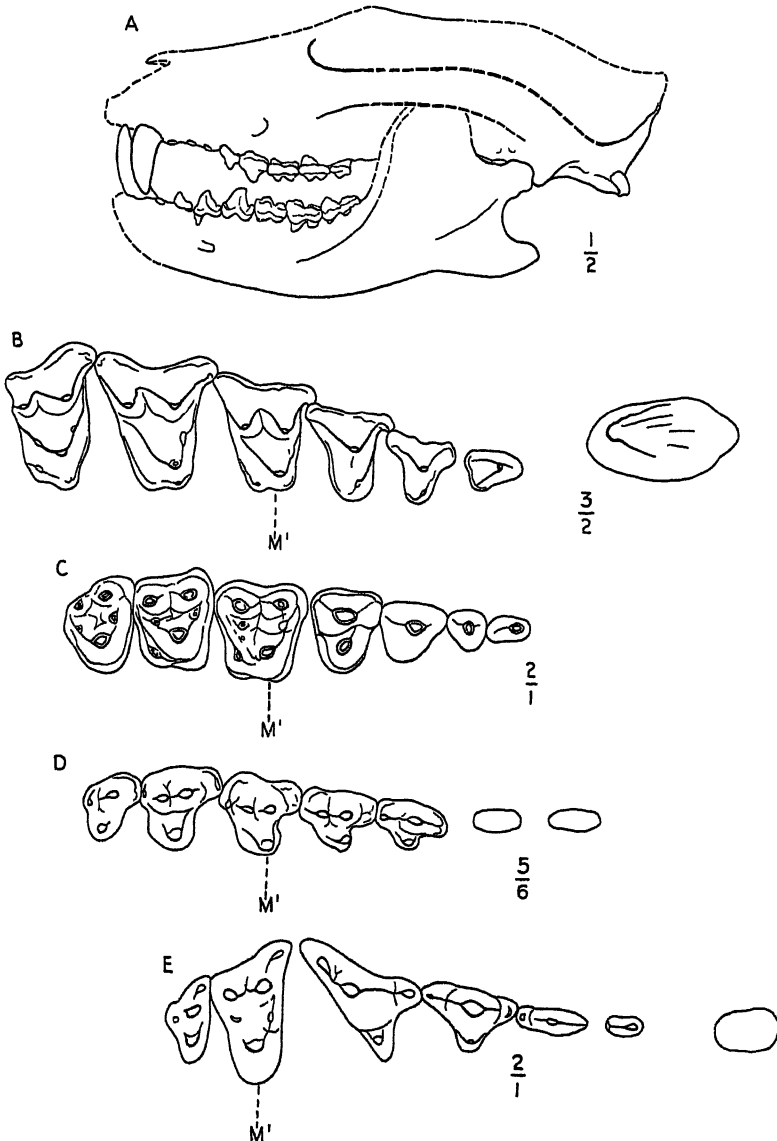


Fig. 6. Paleocene Carnivora. A, *Claenodon montanensis* Gidley, left side view of skull and jaws. B, *Deltatherium fundamini* Cope, crown view of right upper cheek teeth and canine. C, *Tricentes subtrigonus* (Cope), crown view of right upper cheek teeth. D, *Dissacus navajovius* (Cope), crown view of right upper cheek teeth. E, *Didymictis haydenianus* Cope, crown view of right upper cheek teeth. A based on Gidley, B on Gregory, and C, D, and E on Matthew, all redrawn and slightly modified. Enlargements as indicated.

nearly so, brain small, body and limbs slender, tail long, feet plantigrade and pentadactyle, but they present very wide variety in details. Aside from a conservative

generalized group, *Loxolophus*, Puerco, *Chriacus*, Torrejon to Gray Bull, and several other genera, a few more definite adaptive or phyletic trends may be detected. In *Claenodon*, Torrejon, and its allies, culminating in the Lower Eocene *Anacodon*, the molars tend to become broad, flattened, and multicuspid, paralleling the much later development among bears. (As in almost all such cases the convergence has been considered evidence of special affinity, which it surely is not.) In the *Triisodontidae*, represented by *Eoconodon* of the Puerco and *Triisodon* and *Goniacodon*, of the Torrejon, heavy, blunt teeth are developed in which the cusps of the anterior triangles of the lower teeth (trigonids) tend to merge into a single pillar while the upper cheek teeth tend to divide into an inner and an outer pillar. The ancestral tuberculo-sectorial tooth pattern is clearly preserved and little modified in all these forms, and no other type occurs in the Lower Paleocene.

More specialized creodonts began to appear in the Middle and Upper Paleocene. In the Mesonychidae, *Dissacus* in the American Middle and Upper Paleocene (also Lower Eocene) and at Cernay (also in the Sparnacian) carnassials are not developed, but all the lower cheek teeth tend to develop a single anteroposterior row of cusps and the upper cheek teeth have high blunt, conical cusps somewhat as in the triisodonts. The oxyaenids, which develop the first upper and second lower molars as carnassial teeth, are essentially an Eocene group but appear in the Clark Fork. Hyenodontids, with second upper and last lower molars carnassial, were the most long-lived of creodonts, surviving into the Miocene in Asia and Africa. They are probably represented by *Opisthopsalis* in the Gashato, but elsewhere are not known before the Eocene.

The Miacidae, which structurally or literally represent the origin of all the higher carnivores and like the fissipeds have the last upper premolar and first lower molar as carnassials, are represented in the Middle Paleocene by only two genera, the most common of which, *Didymictis*, reappears in the Clark Fork and survives into the Eocene.

Condylarthra. Nothing makes the enormous time that has elapsed since the Paleocene and the fundamental consequent changes in mammalian life more real and impressive than the fact that carnivores and ungulates, so obviously and greatly distinct now, were almost indistinguishable in the Lower Paleocene. It is impossible to say with assurance whether *Protogonodon*, of the Puerco, is a creodont, allied to the claeodonts, or a condylarth, allied to the phenacodonts. Probably it has affinities with both, and its tentative allocation to the Carnivora, as by Matthew, simply follows the necessity to place it somewhere. Similarly all the Puerco species now classified as condylarths have at one time or another been placed in the Creodonta, which they closely resemble. They are placed in the genera *Oxyacodon* and *Tiznatximia*. In the Middle Paleocene the same group, Hyopsodontidae, Mio-claeninae, is represented by *Mioclaenus*, *Ellipsodon*, *Protoselene*, and several other genera. These are all small animals characterized by a curious mingling of proto-carnivore and protoungulate characters, the latter perhaps predominating, with persistently primitive tuberculo-sectorial teeth, the premolars remaining relatively simple and the upper molars trigonal or nearly so through the Middle Paleocene.

More progressive dental characters appear in the Eocene *Hyopsodus*, and *Haplomylus* (the latter also in the Clark Fork), in which the last premolars become submolariform and the upper molars quadrangular.¹ Even the latest members of this group are, however, very primitive in most respects.

The phenacodonts are in general a more progressive group and they are the dominant cursorial or semi-cursorial herbivores of the Paleocene, continuing in abundance in the Lower Eocene but there overshadowed by the perissodactyls and dying out thereafter. The probability that they were derived from a nominally creodont or decidedly creodont-like Lower Paleocene ancestry has been mentioned. In the Middle Paleocene they are represented by the very common *Tetraclaenodon* and a second rarer genus, *Gidleyina*, and in the Upper Paleocene by primitive species

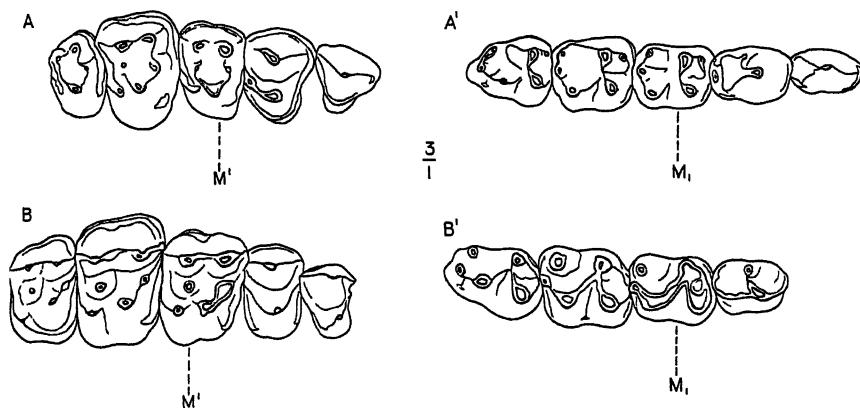


Fig. 7. Paleocene and Eocene hyopsodontid condylarths. A, *Ellipsodon aquilonius* Simpson, Middle Paleocene, crown view of right upper cheek teeth. A', same species, crown view of right lower cheek teeth. B, *Hyopsodus vicarius* (Cope), Middle Eocene, crown view of right upper cheek teeth. B', same species, crown view of right lower cheek teeth. Drawn from specimens. All three times natural size.

of the Lower Eocene genera *Phenacodus* and *Ectocion*. The cheek teeth are persistently brachyodont and are bunodont to sublophodont. During the recorded history of the family they progress chiefly in advancing molarization of the premolars and the addition of accessory cuspsules, eventually becoming polybunodont. The feet are digitigrade, pentadactyl, with some progressive reduction of the side toes. The earliest forms had claws, not unlike those of creodonts, and these later became depressed and are modified into small hoofs.

The meniscotheres are predominantly Lower Eocene mammals in North America, but very fragmentary remains of *Meniscotherium* have been found in the Upper Paleocene. They are condylarths in which the teeth have become markedly selenodont. Two common genera at Cernay, *Pleuraspidothorium* and *Orthaspid-*

¹ One of the curiosities of recent scientific literature is a serious attempt to prove that the insectivore molars arose from a multituberculate quadrangular, and not trigonal, type in which the crucial evidence is the supposed erinaceoid affinities and prototypal character of *Hyopsodus*. Even aside from the demonstrated facts that *Hyopsodus* is not an erinaceoid, or an insectivore, and that the molars do not at all resemble those of multituberculates in structure, the fossil sequence clearly shows its quadrangular molars arising from plainly trigonal molars.

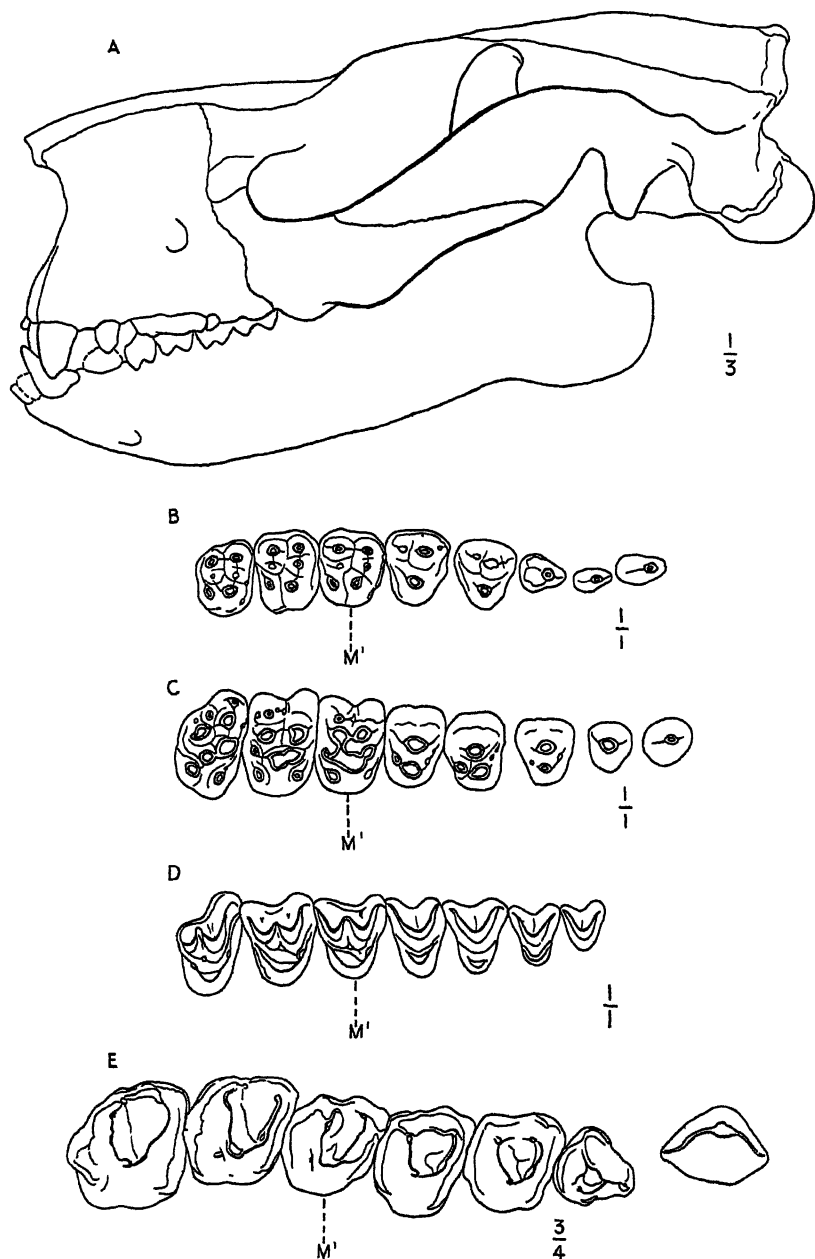


Fig. 8. Paleocene ungulates. A, *Titanoides faberi* Patterson, an Upper Paleocene pantolambdid amblypod, left side view of skull and jaws. B, *Tetraclaenodon puercensis* (Cope), a Middle Paleocene phenacodont condylarth, crown view of right upper cheek teeth. C, *Ectocomus ditrigonus* (Cope), a Lower Paleocene peritychid, crown view of right upper cheek teeth. D, *Pantolambda bathmodon* Cope, a Middle Paleocene pantolambdid, crown view of right upper cheek teeth. E, *Prodinoceras martyr* Matthew, Granger & Simpson, an Upper Paleocene dinoceratan, crown view of right upper cheek teeth and canine. A based on Patterson, B, C, and D on Matthew, and E on Matthew, Granger & Simpson, all redrawn and slightly modified. Enlargements as indicated.

therium appear, following Teilhard, to be meniscotheres, although not very intimately related to the American genus. The group is not known from the European Eocene.

In South America what appear to be condylarths very nearly allied to the phenacodonts appear in some variety in the Eocene Casamayor and one genus, *Ernestokokenia*, also occurs in the Río Chico.

Amblypoda. Although the exigencies and compromises of classification and the history of their study causes the remaining Paleocene ungulates to be united tentatively under the name Amblypoda, this is unsatisfactory and may be misleading unless the real situation, much more complex than can be expressed in a formal classification, is understood. In the first place marked distinction from the condylarths seems to be implied in this arrangement, but the earlier forms of the various groups all approach each other rather closely in many essential respects. The two divisions are distinguishable, and become decidedly different in their later forms, but they are certainly related and when they first appear are unmistakably derivatives of a common ancestry not long antecedent. In a general way the lines classified as Condylarthra are the more slender, semi-cursorial animals, and those classified as Amblypoda are the heavier, rectigrade or subgraviportal animals. In the second place, this sort of division lumps together in the Amblypoda two groups that are not more nearly related to each other than either is to the Condylarthra.

That one of these groups to which the name Amblypoda more strictly applies is represented by *Pantolambda* in the Middle Paleocene, *Titanoides* in the Upper Paleocene, and *Coryphodon* in the Lower Eocene (and latest Paleocene), an ascending structural series although not a perfectly direct genetic phylum. These are progressively larger and heavier animals, becoming of rhinoceros-size, with selenodont cheek teeth later modified by reduction of certain wings on the crescents.

The other group, the Periptychidae, is much more important in the Lower and Middle Paleocene, where it is very abundant, but is represented only by a few fragments of one species in the Upper Paleocene (Tiffany) and is unknown thereafter. Periptychids are varied with five genera in the Puerco and three in the Torrejon, but have fundamentally similar dental characters. The premolars are imperfectly or not molariform and are usually enlarged. The upper molars are basically trigonal but tend toward polybunary. Especially characteristic, although not invariable in the group, is the tendency to develop two small internal accessory cusps, one anterior and one posterior to the inner point of the primitive triangle. In size, there are several small genera, such as *Conacodon*, Puerco, and *Haplocomus*, Torrejon, about the size of hares, and three larger types, *Ectoconus* and *Carsiptychus* in the Puerco and *Periptychus* in the Torrejon and Tiffany, ranging up to the size of sheep. The family represents the earliest, but an unsuccessful, experiment in evolving a varied herbivorous fauna.

At Cernay is found a genus, *Tricuspidon*, that may be related to the amblypods but is too peculiar in tooth pattern and too imperfectly known for any certain conclusion as to its affinities.

Dinocerata. The Dinocerata, or untatheres, large, heavy animals often with horn-like bony bosses on the skull and saber-like canine teeth, resemble the coryphodonts in limb structure and are often classified with them, but the resemblance seems to indicate community of habits and very remote derivation of both from more condylarth-like ancestors rather than any closer affinity. The teeth are very different in the two groups and suggest that the common ancestor must have lived long before either appears in the record (see Simpson, 1929*d*). The untatheres are essentially Eocene animals, but in the Clark Fork and the Gashato appear

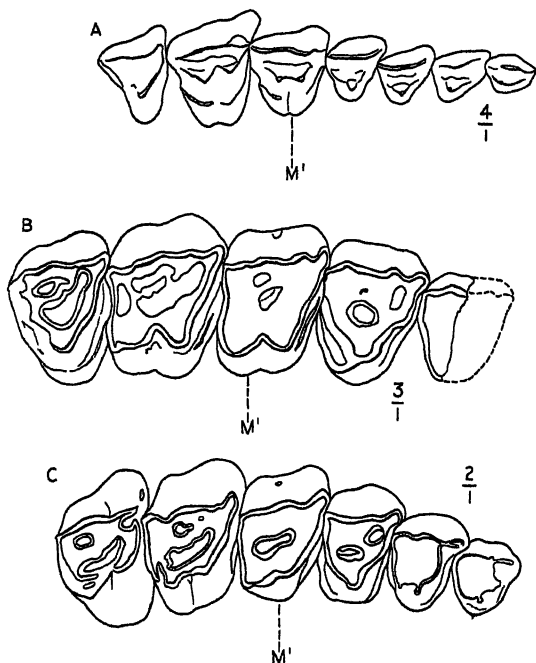


Fig. 9. Paleocene notoungulates. A, *Palaeostylops iturus* Matthew & Granger, from Mongolia, crown view of right upper cheek teeth. B, *Henricosbornia* sp., from Patagonia, crown view of right upper cheek teeth. C, *Kibenikhoria get*, Simpson, from Patagonia, crown view of right upper cheek teeth. A based on Matthew & Granger, C on Simpson, both redrawn and slightly modified. B from specimen. Enlargements as shown.

closely allied genera, *Proathyopsis* and *Prodinoceras*, forerunners of the more specialized Eocene types of the two continents.

South American Ungulates. The Río Chico mammals not previously mentioned belong to the orders Litopterna, Notoungulata, and Astrapotheria. All these, as well as all the families definitely recognized in the Río Chico, survived into later beds where they are much better known and they are not Paleocene as opposed to later Tertiary mammals in the same sense as most of the others discussed here. An apparently paradoxical but fair statement from a different point of view would be that in South America Paleocene mammals characterize the whole Tertiary and are best known from post-Paleocene beds. A reclassification of these groups, with some

notes on their characters and probable relationships, has been given elsewhere (Simpson, 1934), and further research on them is being actively prosecuted. The occurrence of notoungulates in Asia and in North America is discussed elsewhere in this paper.

Artiodactyla. The artiodactyls form a progressive group and they are typically Eocene (and later), not Paleocene, mammals. This fact will not be altered even if it is found that artiodactyls did begin to appear as a sparse vanguard of the coming host in the later Paleocene. *Phenacodaptes* Jepsen, from the American Upper Paleocene has been questionably assigned to this order, but it is more probable that this is a condylarth of the family Hyopsodontidae. Undoubted artiodactyls have never been found in the Paleocene, although the appearance of very primitive precursors there would not be surprising. The ultimate ancestry of the artiodactyls is probably to be sought among early condylarths, comparable to the hyopsodontids, or among the creodonts that most nearly approach the condylarths in structure and are with difficulty separable from the latter.

Perissodactyls have never been found in the Paleocene, and the older view of direct descent from the known phenacodonts has been abandoned. Nevertheless it is very probable that they are the descendants of condylarths and have a more remote, still unknown, common ancestry with the phenacodonts.

VI. PALEOCENE MAMMALS AND THE STRUCTURAL ANCESTRY OF THE PLACENTAL MAMMALIA

Multituberculates constitute a large and important part of the Paleocene faunas, but they are not Eutheria and are so entirely *sui generis* that they have no bearing on the problems here mentioned. Paleocene marsupials, outside South America, are little known and rather close to their modern relatives. The South American mammals are a world apart, as regards relationship to later forms, and cannot be considered in the space here available. Study of the general character of Paleocene mammals refers principally to the Eutheria of North America, with the relatively few Asiatic and European forms also considered as far as they add anything.

From the standpoint of comparative anatomy and of some more general biological problems, the most important question regarding the Paleocene mammals is how far they reveal really primitive or ancestral characters with regard to later mammals or, beyond this, whether the recorded changes in non-ancestral groups are reliably analogous to others in later groups or point back to a still earlier common type of structure.

In evaluating the evidence as to the ancestral characters of placental mammals, there are certain fundamental logical and technical considerations that seem fairly obvious but that are so flagrantly ignored in some recent studies that they merit at least brief and partial statement.

Antiquity is not in itself proof of primitive structure. Obviously some Paleocene mammals are less primitive in certain respects than are some recent mammals. This, however, is no warrant for considering antiquity as of no importance in such discussions, still less does it permit the selection of any evidence from fossils that

seems to support a preconceived thesis and the rejection of all other evidence on the ground that time and sequential factors are not conclusive or important—a favorite procedure with many neozoologists (as opposed to paleontologists or to zoologists in the broader sense) when they bring paleontological data into their arguments.

The more ancient an animal is, the greater the probability that it is more primitive than its later or recent allies. The probability is greater the closer the relationship between the forms compared, until it becomes a certainty if they are ancestor and descendant. Even if the relationship is rather distant, it is proper to assume the existence of this probability unless it can be disproven by cogent evidence free of prejudice.

When an actual sequence in time of closely related forms is known, especially if they are in a single genetic phylum, but even if they are not, and when this sequence shows regular, progressive structural modification, it is proven, for all practical purposes, that the oldest stage in the sequence is the most primitive as regards the structure in question.

In a fauna of contemporaneous animals, if all have in common some one type of structure or show only slight modifications of that structure, it is extremely probable that such a structure is primitive for the smallest taxonomic category inclusive of all the animals concerned.

If the older members of two different groups of related animals resemble each other in some character in which the younger members differ, the type of structure common to the older animals is very probably primitive for both groups.

Characters that are constant through many related animals are much more likely to be primitive, relative to the given stage, or ancestral, than characters that are found in some animals and not in others.

If in two groups known or believed to be of common ancestry a certain type of structure is common to some members of both groups, while other members of each group differ more markedly in this respect from those of the other group, this common type of structure is likely to be primitive for both groups.

In all cases, and particularly in that last mentioned, the nearly universal phenomena of convergence may falsify or confuse the record. The only certain criterion for differentiating between convergence and parallel evolution or divergence from a common ancestry is afforded by actual structural sequences in time. Lacking this, secondary criteria derived from details of the structure in question or, particularly, from the agreement or lack of agreement of other structures with the hypothesis in mind may be almost equally conclusive.

In addition to these elementary principles of research on the origin of anatomical structures, and others that I shall not take the space to list, it is essential to cultivate a time perspective. An example of the necessity is given by a recent study in which certain Upper Eocene primates are taken as more primitive than those from the Middle Paleocene, for the unexpressed but obvious reason that this supports a preconceived theory and with the excuse that all are Eocene (that is, in the old sense, before the Paleocene was distinguished) and hence the probability of being primitive is equal for all. In fact the time interval separating the two is comparable to that

separating the younger of these two from recent animals. The habit of mind underlying such erroneous evaluation of paleontological evidence is that of the comparative anatomist of recent animals, into whose observations no time element enters. The addition of this fourth dimension is one of the peculiarly valuable contributions of paleontology and paleontological data cannot be correctly treated unless its importance is recognized.

Only in small part are the Paleocene placental mammals the actual ancestors of later Tertiary animals. No families and only two orders (Insectivora and Carnivora) survive, and almost all became extinct by the Middle Eocene. No Oligocene or later group can now be traced back directly to one known in the Lower Paleocene. In the later Paleocene there are still no families that survived to the end of the Tertiary, but Primates, Edentates, Rodents, and possibly Artiodactyla, Dermoptera, and Chiroptera (these last three very dubious) are added among surviving orders, and certain families, although defined as distinctive, stand close to or actually are the ancestry of later families, for instance the Anaptomorphidae to the Tarsiidae and possibly other primate families, the Ischyromyidae to some or (in its earliest members) all sciuriforms and perhaps some other rodents, the Miacidae to many or all fissiped carnivores, and the Dichobunidae to various later artiodactyl families.

Despite the limited extent to which actual ancestral forms are known, or at least recognized, in the Paleocene, a great deal of reliable information regarding ancestral structure is provided. Most of the later groups are represented in the known Paleocene faunas not by their ancestors but by allies that had diverged from that ancestry at a time not far antecedent. The structural ancestry is thus well revealed by projecting backward the lines of development within the Paleocene and by examining the characters typifying all or each of various groups there present. Thus in varying degrees and taking each case on its merits as regards the finer points of anatomy, it is a fair conclusion that the primitive insectivore structure is nearly represented in the earliest palaeoryctids and leptictids, that of the primates by the oldest (nominal) anaptomorphids, that of edentates by the palaeonodonts, that of carnivores by the arctocyonids, that of ungulates in general by the amblypods and condylarths and that of perissodactyls and artiodactyls by the phenacodont-mioclaenine-arctocyonid complex. Finally, with so many groups represented in this way, it is a well-supported conclusion that the characters common to all or most Paleocene mammals are primitive for the placental mammals as a whole.

It is impossible here to go into much detail regarding any of the many characters thus revealed as primitive for the various groups or for all, but a few of the more general may be mentioned briefly.

As far as known, the skulls of nearly all Paleocene mammals have long narrow brain cases, the brain itself is small, macrosmatic, and has few convolutions, the rostrum is long and generally slender, the orbits are near the middle of the skull and are open posteriorly, the zygomatic arches are complete and simply sigmoid, and the tympanic is not inflated. Paleocene mammals are quadrupedal with fore and hind limbs of nearly equal length, rather long trunks, and long heavy tails. All are pentadactyl and the earliest members of all phyla, as far as known, are plantigrade

or nearly so. They have divergent and frequently opposable or partly opposable first digits. The early forms, even of those lines that later developed hoofs or nails, have claws, as far as known, or very slightly depressed ungues clearly derived from typical carnivore-like claws.

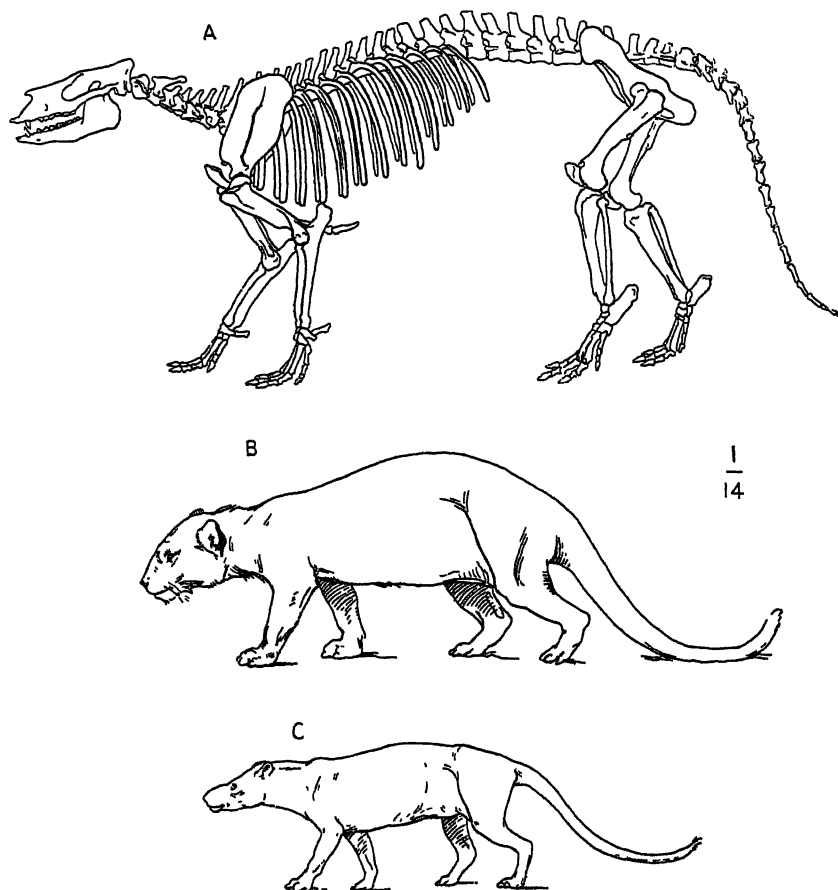


Fig. 10. Paleocene and Lower Eocene ungulates. A, *Phenacodus primaevus* Cope, a Lower Eocene survivor of the typical Paleocene condylarths, left side view of skeleton. B, *Pantolambda*, a Middle Paleocene amblypod, restoration. C, *Tetracaelonodon*, a Middle Paleocene condylarth, restoration. All after Osborn, redrawn. All one-fourteenth natural size.

Regarding these and other skeletal adaptations, Matthew (1904) long since advocated the theory that the Tertiary mammals were derived from arboreal insectivore- or creodont-like ancestors. This view has since been criticized in some quarters and ignored in most as an idle speculation, but it was based on a large body of evidence (some of it still unpublished) and has not been significantly shaken by later discoveries or attacks.

Another of the many interesting points brought out by these data on the generalized ancestral placental mammal is that no real approach to the marsupials

is shown. Early marsupials and placentals resemble each other to the degree that both are primitive within their groups and approach a common ancestral type. But the early placentals show no special marsupial characters in contrast with later placentals. On this and much other evidence it is clear that placentals were not derived from marsupials in any sense more accurate than would also be the opposite statement that marsupials were derived from placentals. They had a common ancestry that was neither marsupial nor placental in a taxonomic sense. ("Marsupial" and "placental" are used as names only: the mode of reproduction of the fossil forms is unknown nor is it as important as the complex of numerous other characters by which the groups to which the names apply are distinguished.)

As regards the dentition, all Paleocene mammals, or their earliest representatives, have brachyodont, heterodont, diphyodont dentitions. With only a few exceptions, obviously specialized in this respect, all early placentals have the dental formula $I_3^3 C_1^1 P_4^4 M_3^3$. The primitive mammals are clearly shown to have had small, simple incisors, fairly large semilaniary canines, simple premolars with little molarization, and trigonal-tuberculosectorial molars.

The last-mentioned point, the ancestral molar pattern, is one on which the Paleocene mammals cast crucial light, and indeed it was from them that this ancestral pattern was first recognized and described by Cope, one of the most far-reaching and fundamentally important generalizations of comparative anatomy. As subsequently developed by Osborn, Matthew, Gregory, and others it is also one of the best founded of zoological generalizations. It is surprising to anyone conversant with the really vast body of evidence that there are still neozoologists who do not accept this theory, although the surprise is somewhat tempered when it is observed that those who do not accept it reveal in their work that they are also those least familiar with the facts bearing on it.

This ancestral molar type has been called tritubercular, in the search for a less clumsy and more comprehensible term than "tuberculosectorial" or "trigonal-tuberculosectorial", but the term "tritubercular" has misled many into thinking that it refers to molars, upper and lower, with just three cusps each. Much time has been wasted belaboring this idea, which is not held by any adherent of the modern tritubercular theory.

Every known Paleocene mammal, marsupial or placental, either shows this pattern unmistakably or can be traced back through a known structural sequence into an older type that does, with the unique exception of the edentates, which already have such degenerate teeth when they first appear, at the very end of the Paleocene, that no cusp pattern is visible.

The lower molars consist of an elevated anterior portion (trigonid) with one outer cusp (protoconid) and two inner cusps (paraconid and metaconid) and a lower posterior portion (talonid) with three cusps on its elevated rim (entoconid, hypoconulid, hypoconid). This is clearly the whole structure of the primitive tooth, and such other cusps as occur are seen to be new structures. In the Paleocene the primitive cusps are always present, although the paraconid is reduced in some lines,

especially frugivorous and herbivorous, and is lost in some post-Paleocene mammals, and the hypoconulid is generally inconspicuous on the first two molars and may eventually be lost there. In some carnivores (notably *Dissacus* in the Paleocene) there is a tendency to lose the metaconid and all but one of the talonid cusps, but in the Paleocene vestiges of the reduced cusps are always visible and the origin from the primitive six-cusped type is plainly shown.

The upper molars are basically trigonal in structure, with three main cusps, one inner (protocone) and two outer (paracone and metacone). There are generally two intermediate cuspules (protoconule and metaconule), which may be primitive in the sense of being possessed by the ancestral placental stock in general, but are always small in the Paleocene and certainly were so in the ancestry, not modifying the ancestral tricuspid nature of the teeth. There is also an external shelf of varying width, probably also primitive, and on it small cuspules may arise, but are so variable that no one can be designated as an ancestral character of all groups.

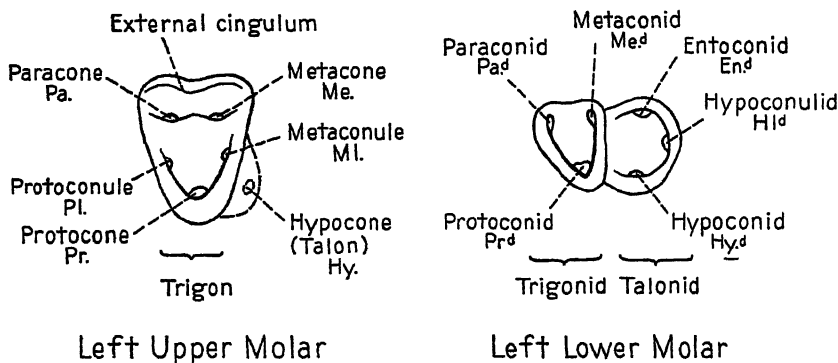


Fig. 11. Diagrams of the generalized primitive upper and lower mammalian molar patterns, with the accepted nomenclature and usual abbreviated designations of the cusps. (The hypocone is a younger cusp and not properly part of the generalized pattern, but it appears in some members of most orders.)

Various other cusps and cuspules are added in some groups, but they vary greatly in the different stocks and in most can be seen to arise within the recorded structural phylum so that all can be positively designated as neomorphs. The most striking modification, and one that has misled many observers not acquainted with the early phyletic stages in which it arises, is the filling in of the embrasures left between the inner apices of the triangular teeth. This is generally by the growth of a postero-internal cusp (hypocone), but in the Paleocene some experiments at filling it in part with an anterointernal cusp (protostyle) are also seen. The evidence that the hypocone is a neomorph is absolutely conclusive, although the details cannot be given in this general review.

For a more detailed consideration of theories of molar evolution Gregory's recent paper (Gregory, 1934) should be read. In considering such theories, as Gregory also emphasizes, a sharp distinction should be made between the theory that the dental type just described is that ancestral for placental mammals (and also for marsupials), a theory so firmly supported that it can rank with little reservation

as an established fact, and the less documented and therefore less certain theories as to the ultimate origin of this type.

The determination of the primitive condition in the teeth and throughout the skeleton is much more important than the immediate point established. The whole conception of the history of mammals, their lines of descent, their relationships to each other, and even many of the more abstract ideas of the modes and causes of evolution depend on the correct identification of the ancestral condition.

VII. RELATIONSHIPS OF CRETACEOUS AND PALEOCENE FAUNAS

It has been marked that the Cretaceous-Eocene transition is marked by the disappearance of the dominant Mesozoic reptiles and the appearance of Tertiary mammals. More precisely, confining attention to terrestrial vertebrates, the reptilian (dinosaurian) orders Saurischia and Ornithischia disappeared and the mammalian orders Taeniodonta, Carnivora, Condylarthra, and Amblypoda appeared in North America, the only continent where Lower Paleocene mammals are surely known. The change in the record is so sudden that it is hardly believable. It is out of harmony with all that is known of other faunal changes (see the remarks on the Paleocene-Eocene transition below) that the old dominant orders should disappear completely and new dominant orders appear suddenly with no overlapping or intergradation, but it is true that nowhere in the world have saurischians or ornithischians ever been found to be contemporaneous with the mammalian orders named or any others characteristic of the Tertiary.¹ The non-evolutionary hypothesis of special creation and perhaps the pseudo-evolutionary hypothesis of magnimutation (to borrow a word coined by W. D. Matthew to describe the idea that major groups arise suddenly by great alterations in structure from one generation to the next) could explain this readily, but neither of these can any longer be considered in sober seriousness. The first truly scientific hypotheses advanced to explain the changes were that a great time gap existed between the known Cretaceous and Tertiary mammal-bearing beds during which the known Cretaceous forms might have evolved into those of the Paleocene, or that the time was short but that the poorly known Cretaceous faunas were nevertheless the source of those of the Paleocene.

Neither hypothesis is now tenable. The gap between the Lance Cretaceous and the Puerco Paleocene in North America is certainly far too short to allow of any great degree of mammalian evolution in the interval. The animals that did survive from one period to the other, champsosaurs, crocodiles, and tortoises among the reptiles and (although the record is less clear and the ancestral and descendant relationship not well established) the multituberculates, marsupials, and insectivores among the mammals apparently changed very little. The floras are closely similar as far as known, so much so that paleobotanists have been prone to place the Fort Union in the Cretaceous because of its Cretaceous floral type. Further-

¹ Only with the Jurassic orders and with multituberculates, marsupials, and insectivores, as mentioned below. The supposed exceptions to this generalization have all been disproven (see *e.g.* Simpson, 1932).

more the structural and stratigraphic relations of the rocks concerned suggest that a nearly complete transition is preserved. Direct derivation of all the Paleocene mammals from the known Cretaceous groups is impossible in any event. The affinities of the Cretaceous forms are now well established, and they all belong to the three orders already named. All three survive into the Tertiary, marsupials and insectivores into the Recent, but after the Cretaceous they occupy a decidedly minor position and never constitute the major portion of any fauna.¹ The insectivores doubtless had a common origin with the dominant Paleocene orders somewhere in the Cretaceous and some are not far removed from that ancestry, but they are not themselves the actual ancestors.

Only two possible explanations remain: that the ancestors of the Paleocene mammals were in fact present in North America in the Cretaceous but have not been discovered, or that they were invaders from some other region. The foremost Paleocene student, W. D. Matthew, was inclined to believe that the first is the essential and the latter a real but a subsidiary factor. It is impossible to prove anything by negative evidence, but the first explanation seems to me highly improbable and almost surely relatively unimportant. The late Cretaceous and early Tertiary deposits of North America are not very different in facies, as is attested not only by their closely similar physical character but also by similarity of flora and of fauna other than dinosaurs and mammals. The known Cretaceous mammals are poorly preserved, it is true, but they are represented by hundreds of specimens from numerous localities scattered over a large area. It is impossible in view of these facts that the difference is caused solely or principally by difference of facies and chances of discovery. Nor does it seem more than a hazardous and improbable guess to suppose that the mammals suddenly changed their habits at the end of the Cretaceous and left an environment where none of their remains were preserved to invade the areas of deposition.

Probably the principal reason for the appearance of so many mammals of types unknown from older beds was immigration into the area where they are found. Where they came from is unknown. Outside North America, Cretaceous mammals are known only from Asia, and there from such a limited area and facies that the possibility that the ancestral Tertiary types were also present is not precluded. The most popular theory is that they did, ultimately, come from Asia. There is, however, no direct evidence and speculation seems futile. The most that can be said is that they probably came from a large land mass that had been separated from North America, by sea or other barrier effective for these animals, for a long time previous to the Paleocene and then was united with it. No such area is definitely known, but one could be surmised in any of several regions of the earth.

Whatever their origin, it is highly improbable that the introduction of these groups into North America was really as sudden as it appears. It will probably prove to be transitional, with some overlapping of range, the dinosaurs disappearing and the mammalian groups expanding gradually from horizon to horizon, when the full record is found.

¹ Outside South America and Australia, not here in question.

VIII. PALEOCENE FAUNAL SUCCESSION

In Europe and Asia the known Paleocene faunas, Cernaysian and Gashato, are each from one small deposit of unit age and no succession, within each continent, is known. In South America the known Río Chico faunules demonstrate with considerable probability the presence of two successive faunas, which I have tentatively designated the *Kibenikhor* and the *Ernestokokenia chaishoer* faunas for descriptive purposes (Simpson, 1935e). In the *Kibenikhor* fauna, apparently older, there seem to be no Casamayor¹ species and most genera are also different from those of the Casamayor. A few genera seem to be the same, but they are identified from imperfect specimens and may well prove to be different. In the *Ernestokokenia chaishoer* fauna, from the very top of the Río Chico, the species are mostly different from those of the Casamayor, although a few imperfectly known forms cannot be distinguished, but the genera seem to be for the most part identical. The data are too few as yet to advance more than a tentative suggestion, but it does seem probable that the Río Chico includes at least two faunas of quite distinct age, perhaps about as distinct as the Torrejon and Tiffany, for instance. A still older fauna (*Carodnia* fauna) may be represented by a few fragments from low levels which are very unlike anything yet discovered in any later horizon, but this is still very dubious. The formation as a whole may well prove to cover much or all of the Paleocene, in which case it will be possible to subdivide it into three or more stratigraphic and faunal entities, but that depends on future work.

The only region where there is a clear and well-known Paleocene faunal succession is Western North America. Here four faunal horizons have so far been identified, each apparently occurring in several different formations or field areas. This sequence has been given on a previous page.

The differences between these (or any other) faunas must be the result of differences in:

- (a) age,
- (b) facies and geographic position,
- (c) chances of collecting.

Directing attention principally to age relations, the best and the only really reliable criterion is the comparison of animals closely related to each other, preferably ancestral and descendent, in different faunas. The frequently more convenient and therefore often more emphasized comparisons by the (supposed) times of the introduction of new groups of animals and extinction of old groups are comparatively very unreliable, since they may be and often are completely falsified by the other factors of facies and chances of collecting.

Applying the more crucial test to the known Paleocene horizons, there are only three known genera which possibly occur in both Puerco and Torrejon: *Eucosmodon*, *Chriacus*, and *Anisonchus*.² The first two of these are known only from inadequate

¹ The Casamayor is a probably Eocene formation which immediately follows the Río Chico.

² Current faunal lists often include others, such as *Triisodon*, *Ellipsodon*, or *Periptychus*, but in all these cases more complete specimens have shown that the specimens from the two horizons are clearly distinct generically, although related to each other.

specimens at least in the horizon not typical for them and may well prove to be distinct. In all three cases the Puerco and Torrejon species are markedly different. In a number of instances approximately ancestral and descendent forms are known from the two horizons, for instance *Wortmania-Psittacotherum*, *Onychodectes-Conoryctes*, *Eoconodon-Triisodon*, and about six other lines. In each case the distinction is marked and is of clear-cut generic value. The orders and families are the same except for the introduction of new groups in the Torrejon. There are no known family extinctions at the end of the Puerco.

Clearly these two faunas are decisively distinct. The unrecorded evolution that took place between the deposition of the fossil levels of the Puerco and those of the Torrejon is considerable, on the average of a grade such as distinguishes decisively separate genera of a successive phylum. A gap in the record is definitely indicated.¹

Comparison of the Torrejon and Tiffany is similar. Five genera seem certainly to run through: *Catopsalis*, *Chriacus*, *Dissacus*, *Didymictis*, and *Periptychus*,² and several others doubtfully identified. The species are distinct in each case, and *Chriacus*, *Dissacus* and *Didymictis* were unusually long-lived genera which survived into the true Eocene. In most lines known in both stages there is a definitely generic advance, for instance *Pronothodectes-Plesiadapis*, *Elphidotarsius-Carpodaptēs*, *Tetraclaenodon-Phenacodus*, and several others. Here again the faunas are very distinct and an unrecorded time interval is indicated. On this evidence, the gap appears to be somewhat less marked than that between the Puerco and Torrejon. It is, however, made somewhat more obvious by the fact that several Torrejon families and lesser phyla are not found in the Tiffany or later beds (*Triisodontidae*, *Mixodectinae*, *Conoryctinae*), and numerous new groups are introduced (*Esthonychidae*, *Plagiomenidae*, *Metacheiromyidae*, *Ischyromyidae*, and others), but these differences are related to facies, migration and chances of collecting, not directly to the passage of time.

The Tiffany and Clark Fork faunas, on the other hand, are closely similar. They have numerous genera in common, such as *Plesiadapis*, *Thryptacodon*, and *Phenacodus*, to mention only a few of the ten or more indicated. In only one case is a generic change suggested within a single phylum, *Carpodaptēs-Carpoolestes*, but even this may be geographic rather than temporal, for the more advanced genus occurs not only in the Clark Fork but also at Bear Creek, in a fauna apparently earlier than Clark Fork and nearly equivalent to the Tiffany.³ The species appear to be generally distinct, but closely allied as far as they belong to the same genera. There are a few changes in supergeneric groups present, the most interesting of

¹ No such gap is visible, or at least has been recognized, in the actual strata, but it certainly is there. The biological evidence is decisive and controverts the negative stratigraphic evidence.

² *Catopsalis* occurs in the Paskapoo, not Tiffany proper, but is there probably at least as young as Tiffany. One or two other genera are not definitely identified in the Tiffany proper, but are found in later beds and so were in existence in Tiffany time.

³ With, however, the distinct possibility that Bear Creek is actually intermediate between Tiffany and Clark Fork or even closer to the latter in time, although very distinct in facies. This possibility, which still appears opposed to the greater probability, was not easily recognized when the Bear Creek fauna was described because then the relationship of Tiffany and Clark Fork was not definitely established.

which is the last known occurrence of *Periptychus* in the Tiffany and first known occurrence of *Coryphodon* in the Clark Fork, but most of these apparent differences may well be correlated with the markedly different facies of the known deposits. The conclusion is that the Tiffany and Clark Fork and their equivalents are essentially continuous with each other and that no important time gap here occurs in the known sequence.

As indicated by the known ordinal and family distribution shown in the following table, the changes in mammalian fauna during the American Paleocene involve not only generic and specific differentiation and advance, as already suggested, but also and most markedly the progressive enrichment of the fauna by the appearance of new groups. Most of the families or larger units persist at least to the end of the Paleocene (and the majority even into the Lower Eocene) once they appear, but each successive stage contains more numerous groups. Thus the Puerco has seven known orders and nine known families, the Torrejon seven orders and nineteen families and the Upper Paleocene (Tiffany, Clark Fork, and equivalents) probably fourteen orders and twenty-nine families.

This progressive diversification is undoubtedly influenced to a considerable extent by the facies of the known deposits, but it is rather surely also in part an original feature of the faunas. It can be explained only in small part, as far as super-generic groups are concerned hardly at all, by evolutionary splitting up of stocks already present in the preceding stage. In greatest part it is probably caused by the immigration of new types of mammals from some as yet unknown center or centers or evolution.

IX. INTERCONTINENTAL RELATIONSHIPS

The first intercontinental Paleocene correlation was made by Cope, who soon recognized the resemblance of his Puerco fauna¹ to that of Cernay. When the Torrejon had been distinguished, it was recognized (*e.g.* Matthew, 1914) that this is closer to the Cernaysian than is the restricted Puerco. Finally, when the American Upper Paleocene had been made known, in part, and the Cernaysian revised, Teilhard (1921) pointed out that the Cernaysian is more nearly equivalent to the Tiffany.² The evidence included the presence of one genus, *Plesiadapis*, with related species (but certainly not identical as Teilhard maintained) on the two continents, the fairly close relationships of certain other genera, such as *Adapisorex* of Cernay and *Leptacodon* of the American Upper Paleocene, the similar general complexion of the faunas, with archaic ungulates and other ancient forms but no horses or adapids, and the closely similar relationships stratigraphically and faunally to the true Lower Eocene, Sparnacian in Europe and Sand Coulee-Gray Bull in America. It would be a refinement unjustified by the evidence to correlate the

¹ It will be remembered that this included what is now called the Torrejon, and that no Upper Paleocene American fauna had then been discovered. Some students, *e.g.* Schlosser, continued to correlate the Cernaysian with the Puerco, not of Cope but *sensu stricto*, long after its much closer relationship to the later Paleocene was established.

² He spoke of the Tiffany as being the extreme upper limit of the Torrejon, which is an indefensible thesis, and did not mention the equally close analogy to the Clark Fork, but his main conclusion was fundamentally sound.

Orders and families of mammals in the American Paleocene and lowest Eocene

	Paleocene			Eocene
	Lower	Middle	Upper	Sand Coulee and Gray Bull
MULTITUBERCULATA				
Ptilodontidae	— 1 —			
Taeniolabididae				
MARSUPIALIA				
Didelphidae				
INSECTIVORA				
Deltatheridiidae ²	— ? —	— ? —		— ? —
Palaeoryctidae ²	— ? —			
Leptictidae				
Nyctitheriidae				
Pantolestidae				
Apheliscidae				
Mixodectidae				
TILLODONTIA				
Esthonychidae				
DERMOPTERA			— ? —	— ? —
Plagiomenidae				
? CHIROPTERA			— ? —	
? Phyllostomatidae			— ? —	
PRIMATES				
Plesiadapidae				
Adapidae				
Apatemyidae				
Carpolestidae				
Anaptomorphidae				
TAENIODONTA				
Stylinodontidae				
EDENTATA				
Metacheiromyidae				
RODENTIA				
Ischyromyidae				

Orders and families of mammals in the American Paleocene and lowest Eocene (cont.)

	Paleocene			Eocene
	Lower	Middle	Upper	Sand Coulee and Gray Bull
CARNIVORA				
Arctocyonidae				
Triisodontidae				
Mesonychidae				
Oxyaenidae				
Hyaenodontidae				
Miacidae				
CONDYLARTHRA				
Phenacodontidae	3			
Meniscotheriidae				
Hyopsodontidae				
TUBULIDENTATA				? 4
AMBLYPODA				
Pantolambdidae				
Coryphodontidae				
Periptychidae				
DINOCERATA				
Uintatheriidae				
NOTOUNGULATA				
Arctostylopidae			5	
PERISSODACTYLA				
Equidae				
Tapiridae				
ARTIODACTYLA				
Dichobunidae			6	

¹ Also in the North American Upper Cretaceous.

² An unpublished Puerco genus (Reynolds), may belong either to the Paleoryctidae or to the Deltatheridiidae, or it may be intermediate between them. The Deltatheridiidae characterize the Mongolian Upper Cretaceous and the Gray Bull genus *Didelphodus* is somewhat doubtfully referred. An ally of *Didelphodus*, *Gelastops*, occurs in the Middle Paleocene.

³ Some of the Puerco forms tentatively referred to *Protogonodon* and placed in the Creodonta may well represent phenacodonts, or their ancestry.

⁴ *Tubulodon* Jepsen, from the Lost Cabin beds, is placed by its discoverer in the Tubulidentata, but the evidence is very incomplete and this may not be its true position.

⁵ Arctostylopids also occur in the Upper Paleocene of Mongolia.

⁶ *Phenacodaptes* Jepsen, from the Fort Union "Tiffany", resembles the primitive Artiodactyla and is tentatively placed in that group by its discoverer, but is probably a condylarth.

Cernaysian with any one subdivision of the American Upper Paleocene, but it almost certainly is of Upper Paleocene age.

Matthew, Granger & Simpson (1929) have pointed out the evidence for the correlation of the Gashato. Briefly it is this: The fauna is of Paleocene type, with multituberculates, creodonts, archaic ungulates, and no modernized groups, but within these ancient groups the genera are advanced and peculiarly aberrant; one genus, *Prodinoceras*, is very close to one from the Clark Fork, *Probathyopsis*;¹ another, *Paleostylops*, is related to but probably more primitive than one from the Gray Bull, *Arctostylops*. The evidence is not conclusive, but it strongly suggests Upper Paleocene age. No more exact correlation can now be made.

Correlation of the Río Chico is still more tentative. It is bounded by a formation (Salamanca) of Senonian or possibly Danian age below, and one (Casamayor) probably of Lower, possibly Middle, Eocene age above. Its upper levels are probably very little older than the latter formation, the difference, as far as known, being analogous to that between Clark Fork and Gray Bull in North America. The lower levels, however, seem to include faunas more decisively older. Its more primitive notoungulates are probably less specialized than *Palaeostylops* of the Gashato or *Arctostylops* of the Gray Bull, but they are on a different line of descent and somewhat more specialized notoungulates are also present. It contains condylarths that are very poorly known but seem to compare approximately with those of the Torrejon, although they could be later. The fauna is decidedly primitive in comparison with later faunas of the same region, but already is distinctly differentiated into several of the major groups typical of South America alone among the continents. Consideration of all these data, against a background of other facts and well tested theories too complex to detail here, suggests that the Río Chico as a whole is Paleocene, although its upper part may extend into the Eocene, and that its better known faunules probably belong in the later half of the Paleocene. There is a distinct possibility that its older levels, with nearly or quite unknown faunas, extend back into the Lower Paleocene.

The following table compares the general faunal constitution of the known Paleocene faunas of the world. Asia is represented by the Gashato, Europe by the Cernaysian, North America by its post-Torrejon Paleocene faunas only (since those are probably more nearly comparable in age with those from other continents), and South America by the whole known Río Chico fauna.

The Cernaysian families are also present in the American Upper Paleocene, except the doubtful Tricuspidontidae. With a single exception (*Plesiadapis*) the genera are different. The faunas of the two continents seem to represent local differentiation from the same general stocks. The difference is, however, striking when it is remembered that in the immediately following Sparnacian and its American equivalent the Gray Bull almost all the genera are the same on the two continents and they have practically identical faunas except for local specific differences. The part played by facies and by the very local nature of the Cernaysian deposit is difficult to evaluate, but it seems inadequate to explain these facts,

¹ A more advanced species of *Probathyopsis* has since been found in the Lower Eocene.

Geographic distribution of Upper Paleocene orders and families of mammals

Name	Asia	Europe	North America .	South America
MULTITUBERCULATA				
Ptilodontidae				
Taeniolabididae				
MARSUPIALIA		?		
Didelphidae		? 1		
Borhyaenidae				
Polydolopidae				
INSECTIVORA				
Leptictidae				
Nyctitheriidae		I		
Pantolestidae		I		
Apheliscidae				
TILLODONTIA		I		
Esthonychidae		I		
? DERMOPTERA			?	
Plagiomenidae				
? CHIROPTERA			?	
? Phyllostomatidae			?	
PRIMATES				
Plesiadapidae				
Apatemyidae		I		
Carpolestidae				
Anaptomorphidae		I		
TAENIODONTA				
Stylinodontidae				
EDENTATA				
Metacheiromyidae				
Dasypodidae				
RODENTIA	?			
Eurymylidae				
Ischyromyidae				

¹ These groups appear in the Sparnacian of Europe, although not yet known in the Thanetian.

Geographic distribution of Upper Paleocene orders and families of mammals (cont.)

Name	Asia	Europe	North America	South America
CARNIVORA				
Arctocyoniidae	?			
Mesonychidae				
Oxyaenidae		1		
Hyaenodontidae	?			
Miacidae				
CONDYLARTHRA	?			
Phenacodontidae		1		
Meniscotheriidae				
Hyopsodontidae				
Didolodontidae				
AMBLYPODA				
Pantolambdidae				
Coryphodontidae		1		
Periptychidae				
Tricuspidodontidae				
DINOCERATA				
Uintatheriidae				
LITOPTERNA				
? Proterotheriidae				
NOTOUNGULATA				
Arctostylopidae			2	
Henricosborniidae				
? Notostylopidae				?
Isotemnidae				
Notopithecidae				
Acoelodidae				
ASTRAPOTHERIA				
Trigonostylopidae				

¹ These groups appear in the Sparnacian of Europe, although not yet known in the Thanetian.

² This family appears in the Gray Bull of North America, although not yet known in the Paleocene.

especially in view of the facial variety of the American faunas. The most probable theory is that Europe and North America received their Paleocene mammals from a common source in the Lower and Middle Paleocene, that they were isolated, or intermigration markedly impeded, in the Upper Paleocene, and that they were again united with free intermigration at the beginning of the Eocene.

An Asiatic Paleocene fauna was sought with great eagerness because it was a widely held theory (especially put forward and documented by Matthew, see 1915*a*) that the new groups of mammals that appear in the later Paleocene and, particularly, Lower Eocene in Europe and North America probably came from Asia and hence that their ancestors would occur in the Paleocene of that continent. The Gashato fauna is very disappointing from that point of view. It does not certainly contain any of the long-sought ancestors. *Eurymylus* is probably a very primitive rodent, but certainly does not represent the ancestry of the whole group. *Prodinoceras* is an ancestral type of uinathere, but it is no more primitive than *Probathyopsis* in America and so leaves open the question as to the direction in which the indicated migration took place. *Palaeostylops* is a possible ancestor of *Arctostylops*, but even in this case there is no proof that Asia was the source and North America the recipient of the group. The other Gashato animals are related in a general way to those of North America and Europe, that is, are of Holarctic type, broadly speaking, but in every case are peculiarly aberrant. They can be visualized only as isolated side branches from the ancestral Holarctic stock, certainly not as belonging to that ancestry.

Too little is known to explain these unexpected relationships with very strong conviction. The facies of the Gashato is apparently unlike that of any other known Paleocene deposit. It may well be that this poorly known and very local fauna is aberrantly specialized for this facies, and that the more generalized, truly ancestral Tertiary Holarctic mammals were, after all, living in Asia at that time but in a different environment.

The South American Paleocene fauna, like all South American faunas before the Pliocene, is almost wholly different from that of the Holarctic continents. It includes no families known from any other continent. It is, however, less distinctive than are the later faunas, and this is a very significant fact. Among marsupials, the earliest borhyaenids are very similar in molar structure, and probably in other respects, to the North American earliest didelphids. The polydolopids are markedly different from anything known elsewhere and represent an aberrant group of unknown antiquity and origin. The earliest armadillos approach in many respects the metacheiromyids which appear in the North American Paleocene, and it seems fairly certain that these two groups had a common ancestry not long antecedent (geologically). The didelodontids, although imperfectly known, are closely similar to the Paleocene and Lower Eocene phenacodonts of North America. They might even be placed in the same family. The earliest litopterns and astropotheres, although already differentiated into these two very characteristic and exclusively South American groups, are not markedly unlike the Holarctic condylarths and it is warranted, as a working hypothesis, to consider them as having been derived from the latter.

Like most South American fossil faunas, that of the Paleocene is dominated, numerically, by the notoungulates. The origin of this group is an interesting but complex problem which cannot be discussed here. Present evidence suggests the tentative view that they, also, arose from a condylarthran or condylarth-like ancestry. In the Río Chico they are decidedly primitive but have definitely acquired all the basic notoungulate characters.

One of the most remarkable facts regarding the distribution of early mammals is the presence of notoungulates in North America in the Lower Eocene and in Asia in the Upper Paleocene. The North American form, *Arctostylops steini*, is known from a single, broken but quite characteristic jaw. The Asiatic genus, *Palaeostylops*, has two species both relatively abundant in the Gashato. Subject to revision from studies now in progress, it appears that *Arctostylops* and *Palaeostylops* are closely related and that they are true notoungulates probably closest to the Henricosborniidae among South American faunas, but that their general structure, or family characters, are not structurally ancestral to all, and perhaps not to any, known South American genera.

When these exotic notoungulates were found, a remarkably clear and simple solution of the geographic history of the groups seemed to present itself. With a very primitive Upper Paleocene form in Asia, a closely allied but more progressive relative in the Lower Eocene of North America, and still more advanced forms in the Middle or Upper Eocene of South America it was almost mandatory to conclude that notoungulates arose in Asia and migrated to South America via North America. This merely illustrates the dangers of dogmatic conclusion from fragmentary evidence, for, although this may possibly have been the history, the evidence now available is by no means so simple and clear-cut and is on the whole opposed to such a history. When *Palaeostylops* lived in Asia, notoungulates were already present in South America and *Palaeostylops* does not represent their ancestry even structurally. It is entirely possible, whatever the ultimate origin of the groups, that *Arctostylops* and *Palaeostylops* represent migrants from, and not to, South America. But the data still are too fragmentary for any positive conclusion.

In any event, the Río Chico fauna shows that South America, or at least Patagonia, was already faunally isolated from Holarctica in the Upper Paleocene and had then already been so isolated long enough for a marked degree of differentiation to have taken place in its mammalian groups. At the same time this fauna does, more nearly than any otherwise known, point back to eventual community of origin with the northern mammals.

X. THE PALEOCENE-EOCENE TRANSITION

Ten or fifteen years ago it was believed that the Paleocene-Eocene boundary was extraordinarily clear-cut faunally, marked by a sudden change not merely in genera or families but in the orders of mammals. It was believed, and accorded with the evidence then known, that at this boundary figuratively in a moment of geologic time, the Multituberculates disappeared and the Primates, Rodentia,

Dinocerata, Perissodactyla, and Arctiodactyla appeared. Partly because of the discovery of intermediate faunas and partly from the discovery of rare forms previously missed in collecting the known faunas, this conception is now much altered. Multituberculates have been discovered in the Eocene (Granger & Simpson, 1928), and Primates (Gidley, 1923), Rodentia (Matthew, Granger & Simpson, 1929; Jepsen, personal communication), and Dinocerata (Matthew, Granger & Simpson, 1929; Simpson, 1929*d*) have been discovered in the Paleocene.

Of the known Paleocene families, only the Taeniolabididae, Carpolestidae, Eurymylidae, Pantolambdidae, and Tricuspidontidae have not yet been found in the Eocene. Of the known lowest Eocene families (outside of South America) only the Adapidae, Equidae, Tapiridae, Lophiodontidae and probably Dichobunidae have not yet been found in the Paleocene. Even in these cases future discoveries may very well extend the ranges into the adjacent epoch.

Particularly in North America, where the Paleocene and Eocene intergrade stratigraphically and almost every stage of the transition has yielded good mammalian faunas, this merely means that the gap in knowledge which gave a clear-cut but purely adventitious boundary line has been almost completely filled. The result is that the apparent line has disappeared and is replaced by an intergradation. This is a more natural state of affairs and any change as abrupt as that which formerly seemed to occur at this point (and still seems to occur at the Cretaceous-Paleocene boundary) must always be viewed with suspicion as more probably apparent than real. The faunal change was by gradual infiltration and gradual extinction of stragglers, not by mass suicide immediately preceding the appearance of a motley host of invaders along a closed front.

Yet attention to these details and recognition of the exact nature of the faunal change should not obscure the fact that the old view was basically correct. Although it was not as rapid as once supposed, the change from Paleocene to Eocene mammalian life was extraordinarily great and sweeping. The difference between typical (not transitional) Paleocene and Eocene mammals is greater than between Eocene and Recent mammals. All the orders (with one or two improbable exceptions) and many of the families of Recent mammals date from the Eocene, but the most typical and abundant Eocene mammals did not belong to the same orders as the characteristic and dominant Paleocene mammals.

The progressive diversification of the Paleocene faunas, already mentioned, was in part caused by the gradual spread of the more modernized groups that were to dominate their spheres of life in the later Tertiary. Similarly the important faunal changes of the Eocene result in part from the continued immigration of such modernized mammals, but they are caused in even greater measure by the gradual disappearance of the old groups doomed to extinction in the ensuing struggle. The typical Paleocene fauna had as its most typical and abundant animals multituberculates, occupying the ecologic niches of rodents, very primitive primates, dominating the arboreal sphere, large fossorial taeniodonts, primitive inadapative creodonts, the flesh-eaters of that epoch, and condylarths and amblypods, the terrestrial, in part cursorial, plant feeders. All of them survived the Paleocene, but

all underwent a very marked diminution in importance at the beginning of the Eocene and, with the exception of a few obscure archaic primates and insectivores that escaped extinction in outlying tropical areas and a few very specialized creodonts that survived a little longer than the others, none survived to the end of the Eocene.

The general view as to the relationships of the "archaic" Paleocene mammals and the "modernized" Eocene mammals here set forth differs from both of the two most noteworthy previous considerations, those of Matthew and of Osborn. Although both treat the subject circumspectly and do not draw decisive conclusions from the indecisive evidence, it is fairly clear that Matthew (for instance, 1929, also in many other papers) considered the difference to be very largely geographic, with the differentiation of generally similar groups of mammals along two lines, one, earlier and perhaps in North America, into the Paleocene orders, the other, later and probably in Asia, into the Eocene orders. In his earlier work (he has not recently specifically reviewed this problem) Osborn inclined to the view that both faunal types originated in the same area, Asia, but that they arose by a primary dichotomy of the Eutheria into Meseutheria, the archaic types, and Ceneutheria, the modernized types (see, for instance, Osborn, 1905). It now seems somewhat more probable that a primary multiple division into carnivorous, herbivorous-ungulate, arboreal-frugivorous, etc., stocks took place somewhere, perhaps Asia although the evidence is very poor, that the Paleocene faunas are a first radiation from this multiple group, and the Eocene faunas a second radiation after the same separate basic stocks had undergone further and more progressive evolution. Obviously all three opinions, none of which is adequately expounded by such brief statement, have much in common, including all that is really fundamental, but they involve distinctly different viewpoints.

The foregoing remarks are based primarily on the North American conditions and apply equally, as far as the more scanty data go, to Europe and Asia. In South America the condition is very different. All the known South American Paleocene families survive into the Eocene. Most of the orders and several of the families (either directly or through derivative families) survive into the later Tertiary, and indeed most of the orders are still present in the Pleistocene. There is no such marked change, either abruptly or transitionally, between the South American Paleocene and Eocene as exists between these epochs in North America, but only a fairly continuous development of the fauna into the Pliocene.

In sharp distinction from Holarctica, then, the South American Tertiary faunas (with the possible exception of a few elements, notably rodents) are simply a development, *in situ*, of the Paleocene fauna. The change in South America which corresponds in kind with that between the Paleocene and Eocene in the north took place very much later, at about the Pliocene-Pleistocene boundary. The parallel is very close and is enlightening. As in the transition discussed above, the change in South America was gradual. Well down in the Pliocene a few progressive immigrants began to filter into southern South America, and a number of the archaic native mammals survived into the Pleistocene, but near the Pliocene-Pleistocene boundary they are markedly reduced in variety and abundance, and very few of them

survived the Pleistocene. In South America the descendants of the archaic Paleocene fauna met modernized invaders from North America and succumbed to them at the end of the Tertiary. In North America the invaders appeared around the end of the Paleocene, and it is not known whence they came, but the phenomena are essentially the same in both cases.

XI. SUMMARY OF MAMMALIAN FAUNAL HISTORY

The oldest known mammals, from the Rhaeto-Lias in Europe and Africa, do not include the ancestors of the later mammals and have little bearing on mammalian faunal succession (see Simpson, 1928*b*). The Middle Jurassic fauna of England and the Upper Jurassic faunas of England, the United States, and East Africa (one specimen) are of a distinctive faunal type and suggest that this sort of mammalian fauna had then spread over a large part of the world. They include multituberculates, triconodonts, symmetrodonts, and pantotheres (Simpson, 1928*c*, 1929*c*). The multituberculates reappear, in more advanced and varied form, in the Cretaceous and Lower Tertiary. The triconodonts and symmetrodonts do not reappear and probably became extinct during the early Cretaceous. The known pantotheres seem to represent a Jurassic radiation from the common marsupial-placental stock.

The known Upper Cretaceous faunas also are of a distinctive faunal type, but one quite different from that of the Jurassic. They consist of multituberculates (Asia and North America), marsupials (North America), and very primitive placentals of rather undifferentiated insectivore-carnivore type, classified as *Insectivora* (Asia and North America) (Gregory & Simpson, 1926; Simpson, 1928*d*). The latter apparently represent primary dichotomous differentiation of the general pantothere stock, with a secondary local radiation within each group.

The known Paleocene faunas of North America, Europe, Asia, and South America probably all had a common source and represent the radiation of a fauna derived from one of the known Cretaceous types but much more highly differentiated. Among the multituberculates and marsupials this differentiation was of relatively minor grade, in taxonomic terms of family or at most subordinal rank, while the more progressive and adaptive placentals show the beginnings of a more profound splitting, ultimately of ordinal rank,¹ and are more numerous and varied. In North America, at least, this new faunal type appears as an invasion from some unknown evolutionary center.

In Europe, North America, and Asia, a new type of fauna began to appear during the Paleocene, the change culminating at the end of the epoch and becoming entirely complete during the Eocene. The new fauna is less markedly different from the old than in the previous changes noted, and consists of the appearance of new or "modernized" groups clearly derived from an already partly differentiated fauna of Paleocene type. The new forms appear to be immigrants where found, and came from some unidentified area where the earliest Paleocene fauna was well developed

¹ This, however, is in part based on our knowledge of the destinies of these groups. They were in fact still quite closely related in the Paleocene, especially the older Paleocene, and it is probable that they could all properly be classified as one order were no later placental mammals known.

and where its rapid and diversified evolution was permitted and stimulated. Only placental mammals were involved, the few surviving multituberculates and marsupials clearly being stragglers from the known Paleocene.

There has not been any other major spread of mammals or great change in faunal type. With positive changes resulting from long evolution and from repeated intermigration and negative changes resulting from extinction, the mammals now peopling at least the Holarctic continents are essentially those that appeared there in the Eocene invasion. In South America this change was long delayed, and what is essentially the incursion of the Holarctic Eocene fauna into the previous habitat of the Paleocene fauna took place at the end of the Tertiary and not toward its beginning as in Holarctica. In Australia this change never took place (aside from the agency of man). The early faunal history of Africa is unknown and still beyond logical conjecture.

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ADDENDUM

While this paper was in the press, the publications listed below have appeared, and research on the Paleocene has been actively continued. Much of this work was known and, in a general way, embodied in the present manuscript, but a few additional comments are necessary to bring this review up to date as of November 1936.

The proposal of the name "Plateau Valley beds" for the Paleocene part of the formation formerly called "Ruby" has been mentioned in a footnote. Wherever the name "Ruby" appears, "Plateau Valley" should be read.

The possible Lower Paleocene deltatheridiid mentioned on the authority of Father Reynolds has now been published and is *Puercolestes* Reynolds, 1936.

Dr Jepsen informs me (personal communication) that he now considers his genus *Phenacodaptes* to be a condylarth, a conclusion already suggested in the foregoing pages. In view of this agreement, it is fair to conclude that no probable artiodactyl is yet known from the Paleocene, and the statement of this as possible (but not probable) may be removed from the record. Dr Jepsen has in hand a large and very important Paleocene collection from Wyoming and also stratigraphic studies that will give more definite names and data for the stratigraphic subdivisions in that area, but this work has not been published and is not promised for the immediate future, so that no more explicit reference to it can now be made.

Dr A. E. Wood states (personal communication) that *Eurymylus* cannot be a rodent and probably is not a lagomorph. In view of his excellent acquaintance with fossil rodents, this opinion carries great weight, but, pending a more detailed analysis of the evidence, it may not be quite conclusive.

Description of a new fauna from a high level in the Fort Union group of the Crazy Mountain Field (Simpson, 1936 c, below) shows these beds definitely to extend at least to the early Upper Paleocene. The primitive characteristics of the mammalian dentition have been described in some detail (Simpson, 1936 d) and the name "tribosphenic" proposed to characterize the molar type more ambiguously and clumsily called "trigonal-tuberculosectional". The revision and description of the National Museum collection from the Fort Union of Montana has been completed and is now in the press. Most of its results were known when the present manuscript was prepared. One important change in classification is made by transferring the Peripitychidae to the Condylarthra, a formal change less radical than appears at first sight, in view of the anomalies in the current

arrangement already mentioned in the preceding pages. The Fort Union group of the Crazy Mountain Field is somewhat altered in nomenclature, as follows:

Former designation	New designation
"Upper Fort Union", or "Fort Union No. 3"	= Melville
"Lower Fort Union", "Fort Union Nos. 1 and 2", or "Lebo"	= Lebo
"Tullock", or "Upper Lance"	= Bear

The paper on the Clark Fork listed above (as Simpson, 1936) is still in the press, but its conclusions were available for the present paper.

A very large new collection from several different horizons in the Fort Union is now in hand, but completion and publication of research on it will take some months. As far as can now be foreseen, it will contribute greatly to knowledge of details and within limited groups, but will not significantly alter the more general outline of the Paleocene given here.

The very important memoir by the late W. D. Matthew on the Paleocene of the San Juan Basin is now in the press and will be published in the immediate future in the *Transactions of the American Philosophical Society*. Its conclusions were available in preparing the present review.

- PATTERSON, B. (1936). "Mounted skeleton of *Titanoides*, with notes on the associated fauna." (Abstract.) *Proc. Geol. Soc. Amer.* for 1935 (published 1936), pp. 397-8. [Proposes the name "Plateau Valley beds" for the Paleocene part of the formation hitherto called "Ruby".]
- REYNOLDS, T. E. (1936). "Two new insectivores from the Lower Paleocene of New Mexico." *J. Paleont.* 10, 202-9. [*Puercolestes* and *Escatepos*, the latter a doubtful creodont.]
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BLOOD SUBSTITUTES

BY WILLIAM R. AMBERSON

(Department of Physiology, College of Medicine, University
of Tennessee, Memphis, Tennessee, U.S.A.)

(Received 24 January 1936)

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I. INTRODUCTION

THE blood of vertebrates is the most complicated fluid to be found in the world of living organisms. Compounded of a dozen essential ingredients, sustaining a multiplicity of activities, the fluid pathway for a variety of chemical and hormonal integrations of function, the source of food and oxygen for every tissue, it defies laboratory synthesis. At the very beginning we must recognize that there is no complete substitute for blood. Yet biologists and physiologists, no less than clinicians, are so frequently confronted with situations where normal blood cannot be obtained, or where the problem at issue can only be solved by a simplification of conditions, that a substitute for blood has become one of the most pressing needs of the experimental laboratory.

It is our purpose, in this review, to consider some of the more important developments in theory and practice in this field of study, with particular reference to the mammalian body. The review will definitely not deal with the transfusion of whole blood from one animal to another (see Doan, 1927, for review), nor even with interspecies transfusions of whole blood (see Cruchet, Ragot & Caussimon, 1928; and Kunz, 1929); neither will it consider, except incidentally, the problem of ion balance or acid-base regulation (see Dittler, 1922, for review), the principles of which are so widely appreciated that an acquaintanceship with them, on the reader's part, will be taken for granted. We will have to do, rather, with fluids which depart significantly from whole blood in their composition, which contain, at best, only a part of normal blood, or which, at worst, have none of its normal constituents.

When hemorrhage has been slight or moderate the mammalian body is able to draw quickly upon the tissue fluids, which pass into the blood stream under the

influence of the normal blood colloids, as the blood pressure is somewhat lowered. Tissue fluid, therefore, is the normal or natural blood substitute, quickly supplemented by more red cells and plasma proteins from their respective reserve depots. For a review of the literature covering such rapid, natural replacement of small losses in blood volume the reader is referred to the recent paper of Robertson (1935).

We will here be concerned, however, with more extreme conditions, where the capacity of the normal replacement mechanism has been exceeded, or where we are interested in maintaining the activities of isolated organs. In the lower vertebrates the blood may be completely removed and replaced by salt solutions. The original "Salzfrösche" (Kühne, 1859; Cohnheim, 1869; Tiegel, 1876; Oertmann, 1877) had their blood washed out by long perfusion with a pure sodium chloride solution, and survived for some hours. Von Moraczewski (1899, 1900) prepared "sugar-frogs" and "urea-frogs" as well, and found that they could live for days if only a little of the original blood was left in the body. More recently salt-frogs have been more carefully prepared by Gayda (1921) and especially by Ferrari (1929, 1932 *a, b*), who has been able to get these animals to survive indefinitely, after perfusion with Ringer solution until an original normal blood cell count of 400,000 per c.mm. was reduced to 3000–6000. After such treatment the plasma proteins return to normal in about 60 hours, but the recovery of the red cells is very slow, reaching only 20,000–40,000 after four months. Survival under these conditions is perhaps not so surprising when we remember that reflex action can continue for some time in completely isolated frog central nervous systems without any circulation whatsoever (Baglioni, 1904).

Even the mammalian heart continues to beat for some time if perfused with oxygenated Ringer-Locke solutions at mammalian temperature (Martin & Applegarth, 1888; Langendorff, 1895, 1902, 1905; Hooker & Kehar, 1933; and many others). It quickly develops edema, however, and is almost certainly anoxic.

It is impossible to maintain life in the whole mammalian body after the complete replacement of the normal blood with any salt solution. No matter how ingeniously contrived such solutions remain in the blood stream for a short time only, whether or not there has been a prior hemorrhage. They pass quickly into the tissue spaces in various organs (Miller & Poindexter, 1932), of which the liver appears to be the most important (Lamson & Roca, 1921; Skelton, 1927; Krogh, 1929; Roberts & Crandall, 1933). They are unable to restore lost blood volume and raise blood pressure for more than a few minutes. It is true that certain salt solutions without colloidal components can exert a favorable physiological influence, but their effect results more from their pharmacological action upon the heart and blood vessels or upon the pH of the blood than from any real ability to replace normal blood. Among such solutions may be mentioned Normet's fluid (L. Normet, 1925, 1929, and M. Normet, 1929, criticized by Giraud & Silhol, 1930), in which the addition of sodium and other citrates to Ringer-Locke stimulates the heart and produces vaso-constriction, and Hartmann & Senn's (1932) lactate-Ringer, where sodium γ -lactate is added to Ringer-Locke, and is gradually converted into sodium bicarbonate within the body, so that an uncompensated alkalosis is avoided.

Glucose-saline solutions are also frequently beneficial, as Pal & Prasad (1935) have again shown for the perfused frog heart, but even hypertonic sugar and salt solutions leave the blood vessels within a few hours at the most (Kinsman, Spurling & Jelsma, 1928; Watanabe & Kurokawa, 1929; Wierzuchowski & Pieskow, 1930). Amino-acids added to saline solutions may exert favorable influences, as for example the addition of glycocol to solutions for frog kidney perfusions (Höber, 1932, 1934), but when infused into the mammalian body these acids leave the blood stream almost at once (Schlossmann, 1926; Wiechmann, 1926). Urea is also known to exert a beneficial effect, but similarly leaves the blood in a few minutes (Nonnenbruch, 1921). Even injected fat is said to leave the blood stream within 10 min. (Del Baere, 1926).

In all fairness to the literature, however, we must recognize that a few workers still look upon isotonic sodium chloride as an acceptable blood substitute. It is still possible for Hoitink (1935) to conclude that for rabbits, after hemorrhage, such a solution is just as good as any other, save whole blood alone. Yet simultaneously Konrich (1935) arrives at exactly the opposite conclusion on the same material, finding that pure sodium chloride solution is inferior to all others. We are obviously entering a field where considerable differences of opinion are possible.

If solutions containing only crystalloids cannot be accepted as blood substitutes our discussion obviously resolves itself into a consideration of fluids containing organic and colloidal constituents which are able to maintain the colloidal osmotic pressure of the blood and so restore blood pressure and blood volume in a more permanent manner. The significance of the blood colloids in maintaining a normal water balance was clearly appreciated as early as 1859 by Bernard, but the classic work of Starling (1896) led to our present recognition of their importance. As a result of Starling's work we now recognize that, although the blood crystalloids pass with ease, the much larger molecules of the plasma colloids, mainly protein in nature, escape through the capillary walls with considerable difficulty. In consequence there is normally a protein gradient across these walls; as a result of this gradient there is an effective colloidal osmotic pressure, often now referred to as the "oncotic pressure" (Schade & Menschel, 1923; Schade, 1927) which tends at all times to draw water from the tissue fluids into the blood stream. At the arteriolar end of the capillaries the blood pressure is higher than the oncotic pressure, so that fluid leaves the blood stream, whereas at the venous end of the same vessels the blood pressure has fallen below the oncotic pressure, so that water moves back into the vessels once more. Fluid interchange between blood and tissues is thus facilitated, and blood volume maintained.

Certain colloidal materials which have been proposed for use in blood substitutes may be dismissed with a word. The higher protein derivatives such as peptone may have a stimulating action on isolated perfused tissues but in the whole mammalian body they produce a shock-like condition with a great fall of blood pressure (Heidenhain, 1891; Underhill & Ringer, 1922; Cloetta & Wünsche, 1923; Foa, 1925; Stuber & Lang, 1930; and many others). Milk infusions, even into the human body, once had a considerable vogue (Thomas, 1878; Jennings, 1885;

Guthrie & Pike, 1907), but many failures and the development of blood transfusion have led to the discontinuance of this method.

Our study of the literature leads us to the conclusion that the fluids which may now be considered as blood substitutes fall into the following categories: (1) blood plasma and serum, (2) hemoglobin-Ringer and hemolysed blood, (3) gum-saline, and (4) gelatine-saline.

II. BLOOD PLASMA AND SERUM

We shall first consider the problem of the substitution of blood serum for whole blood, avoiding so far as possible the whole field of serology and anaphylactic reactions, and considering mainly the availability of homologous plasma or serum for infusion and perfusion work. The record opens in the laboratory of Carl Ludwig, where Bowditch (1871) and Luciani (1872) filled frog's hearts with sheep and rabbit serum, observed the development of arrhythmia as the serum aged, and the restoration of the normal beat with new serum, and where, a little later, Sedgwick-Minot (1876) successfully perfused m. biceps and semitendinosus of dogs with dog and calf serum. In the same period Kronecker & Stirling (1875) and Merunowicz (1875) inaugurated a long series of studies directed to the determination of the constituent of serum which is most important in such work. Their fundamental observation was that a frog or turtle heart, perfused with pure 0.6 per cent. sodium chloride, soon became quiescent, but could have its beat restored by the use of blood or serum. Using this technique for testing the importance of various serum constituents, Merunowicz tested the effect of solutions made up from various serum fractions. He was led finally to believe that the sodium bicarbonate content of serum is of prime importance in its beneficial effect, and reported good results with 0.6 per cent. sodium chloride plus a small amount of the bicarbonate.

Three years later Gaule (1878) continued this work and reported that the effect was not dependent upon bicarbonate itself but was due in part to the alkalinity of the solution, since he could get recovery when 5 mg. of sodium hydroxide were added to each 100 c.c. of sodium chloride solution. He made alkaline extracts of "warm" and "cold" hearts (*i.e.* from animals previously in the warm or in the cold) and found that such extracts gave a vigorous heart beat typical of a "warm" preparation even when perfused through a "cold" heart. He searched for the substance responsible in the extract, and concluded that it was a peptone-like material, since an alkaline peptone solution gave a similar effect on the heart beat. He was led to stress the importance of an organic factor, as well as the alkalinity. In the same year Stienon (1878) confirmed the importance of an alkaline reaction by showing that heart recovery was more difficult in bicarbonate-free serum. He cooked serum, and found that the fluid expressed from the protein coagulum was less effective in restoring the heart beat than was fresh serum itself. He concluded with Gaule that an organic constituent of serum, soluble in water or dilute alcohol, and probably a protein, was necessary for full and quick recovery.

Martius (1882) also found that an alkaline sodium chloride solution can restore

the heart beat, but secured only transient restorations, with a second cessation of beat which the same solution could not restore. He was unable to get heart recoveries with peptone (as Gaule had believed possible) and also failed with glycogen, syntonin, egg albumin, milk casein and myosin. Lymph, however, was as potent as whole serum. He concluded that the serum albumin is the organic constituent responsible for heart recovery. Similar conclusions were reached by von Ott (1883) who failed to get heart recovery with globulin, paraglobulin, or Schmiedeberg's crystalline protein. He fed dogs cheese, peptone and similar substances, and made up perfusion solutions from the liquid portions of the partly digested material, finding them able to recover the completely fatigued heart. He naively concluded, from this and similar experiments, that serum albumin was manufactured in the stomach.

It is beyond the limitations of this review to consider in detail the further studies of this school of workers. The main conclusions were confirmed by Kronecker & Popoff (1887), Brinck (1889), Popoff (1889), Schücking (1901), Finn (1906), Algina (1908), Lussana (1908 *a, b*). More comprehensive reviews of this old literature are given by Langendorff (1902, 1905) and Tigerstedt (1912).

Although at first Ringer (1882) dismissed the idea that serum albumin is important, he later (1885) accepted the view that a balanced salt solution is not enough to maintain the heart beat, finding that, particularly in the breeding season, the beat of the perfused heart ceased relatively early with salt solutions but could be restored by the addition of small amounts of blood or serum. He also found that (contrary to Kronecker and others) egg white was quite effective in restoring the beat and that even gelatine, although inferior to serum, had a considerable effect. In spite of Ringer's acceptance of the importance of a protein constituent later investigators have insisted that all of the earlier results were due to the attainment of proper salt balance in previously unbalanced solutions (Howell & Cooke, 1893; Walden, 1899), and it must be recognized that all of the work prior to Ringer was inadequately controlled in this respect. Walden perfused the frog heart with sodium chloride solutions containing serum-albumin, but found it absolutely incapable of maintaining the beat. Only balanced solutions were able to do so.

This line of research may be considered to end in the papers of Gorham & Morrison (1910) and Wieland (1921). Using Newell Martin's method, the former authors, under Howell's direction, perfused the isolated cat heart with solutions containing fibrinogen, albumin and globulin. Contrary to the claims of Kronecker's school they concluded that "the several proteins used in perfusing the heart...have no distinctly favorable action in sustaining the heart beat". They did, however, observe a gradual disappearance of fibrinogen and of globulin from their recirculated solutions, indicating their use in some way by the heart, whereas albumin tended to increase. Unfortunately they used concentrations of protein so low (0.1-0.2 per cent. only) that it is not clear that their experiments were comparable with those of the earlier workers, particularly if we accept the opinion, which will be further developed as we proceed, that it is the physical properties of the blood colloids which are important in this work, rather than a chemical influence.

Wieland developed the interesting idea that the hypodynamic state is a sort of poisoning, and working on this hypothesis added not only serum, but such surface-active agents as sodium oleate, ether, xylol, camphor and animal charcoal to his perfusion fluids. He found that such agents were able to restore a normal beat to the hypodynamic frog heart, and believed that they did so by adsorbing and thus detoxifying the accumulated products of metabolism within the heart. His work certainly raises grave doubts concerning any specific or unique value of the serum proteins in perfusion solutions.

In its modern form the protein theory has found new expression in such studies as those of Whipple, Smith & Belt (1920) and of Belt, Smith & Whipple (1920), who, observing shock in dogs after the removal of a portion of the serum protein, concluded that the "blood serum proteins are stabilizing or protective factors"; and of Ellinger & Heymann (1921) who found that edema did not occur so readily in the legs of frogs when perfused with Ringer to which mammalian serum had been added; of Drinker (1927) who observed capillary dilatation and edema in the same preparation unless some serum was present; and of Smith & Dick (1932) who increased the blood proteins in rabbits by injection of a heparinized plasma-protein concentrate, and found that dyes left the blood capillaries more slowly, whereas, when the proteins were reduced by plasmapheresis dye escape was greater. This literature and other relevant papers are reviewed by Drinker & Field (1933). They conclude that the serum proteins act to control the permeability of the capillary wall in some other way than by their regulation of colloidal osmotic pressure alone.

The weight of evidence, however, seems to us to favor a physical rather than a chemical interpretation of the major significance of the plasma proteins. The importance of their colloidal osmotic pressure has been re-emphasized by the use of the technique of "plasmapheresis" introduced by Abel, Rowntree & Turner (1914) in which the plasma is depleted by removal of blood and reinjection of the washed cells resuspended in Locke solution. By this method or by the production of nutritional edema (Weech, Snelling & Goettsch, 1933), the physiological consequences of low blood protein may be studied, and the basic validity of Starling's theory of water balance confirmed. In the most recent use of this method Holman, Mahoney & Whipple (1934) and Pommerenke, Slavin, Kariher & Whipple (1935) have shown the importance of dietary factors in blood protein regeneration, and have made the significant observation that injections of dog plasma will maintain a protein-fasting dog in nitrogen equilibrium, whereas the serum of another species cannot so act. We thus have a modern confirmation of the old view that the serum proteins enter into the activity of tissues in a unique manner, being directly available to the living cells for their growth and metabolism. It does not, however, follow that in order to maintain life they must be continually present in the body since, as we shall see, they may be almost completely removed, at least for short periods.

In their search for the constituent of serum or plasma which is most significant, a smaller group of students have held that it is the lipoidal constituents rather than the proteins which are responsible for the beneficial effects. Ringer (1885), finding that ether extracted blood retained its power to restore the beat of the heart rendered

completely quiescent by long sodium chloride perfusion, concluded that "the effect... is not dependent on the fats...". Later workers have reached a different conclusion. Danilewski (1907*a*) added lecithin in low concentration (0.002–0.05 per cent.) to Ringer solution and found it powerfully effective in restoring or maintaining the beat of the excised frog heart. In a later paper (1907*b*) he reported that cholesterol had a similar, though less marked, effect. Clark (1913) reviewed other literature bearing on the point and reported that, while serum is markedly beneficial, serum proteins, free from lipid, have no effect in recovering the hypodynamic heart. He prepared alcohol and ether extracts of serum and, contrary to Ringer, found the alcohol or ether-soluble fractions to contain a substance capable of restoring the heart beat. He also found lecithin, sodium oleate, and other lipoidal substances to be effective. The beneficial influence of sodium oleate was confirmed by Wieland (1921). Van Leeuwen & Szent Györgyi (1923) found that pure lecithin is without effect, and ascribe the results in Clark's experiments to the presence of considerable cephalin, which, in pure form, has a marked ability to recover the heart. It also lowers mammalian blood pressure, whereas lecithin has no effect. Some recent investigators (Dresel & Sternheimer, 1928; Ziganow, 1926; Ueda, 1930; Kimmelstiel, 1932) have confirmed Clark's work, in general, although Kimmelstiel finds cephalin toxic, and reports that hydrophobic lipoids, such as cerebrosides and cholesterolin, have no direct effect.

Eggleton (1926), using carefully purified preparations of lecithin and cephalin, reported that "neither phosphatide was found to be a cardiac stimulant", and believed that the activity ascribed to them was really due to a water-soluble impurity, possibly an organic base. Burrige (1928) believed that cholesterol and lecithin exert two actions on the heart: (1) a depression due to interference with calcium function, and (2) an augmentation due to colloidal changes, with the second effect dominant at low concentrations, the first at high. Since serum has only an augmenting effect he reasons that the process of extraction must produce depressor substances, and doubts whether previous investigators were studying the effects of lipoids in their natural state.

From this review we must conclude that our ideas about the significance of the organic constituents of plasma in perfusion and infusion work are still in a rather unsatisfactory state. Practically all students now recognize the importance of an organic factor, but essential disagreements persist concerning the identity of that factor and its mode of operation. We feel that the plasma colloids, both protein and fat, probably exercise their major effect by maintaining the colloidal osmotic pressure of the blood. As we shall see later, the normal colloids may be almost completely replaced by other colloids, without injury to the mammalian body, *if there be no oxygen lack*. Oxygen lack undoubtedly occurred in many of the experiments cited above, and the literature is in a state of some confusion because of failure to control this and other factors.

Practical difficulties in the use of blood serum as a substitute for whole blood arise out of the appearance in it of chemical substances which are toxic or which exert a vaso-motor effect. Strictly fresh defibrinated blood or blood serum is often

extremely toxic (see Moldavan, 1910, for literature), producing intracardial and intravascular clotting. The effect vanishes in about half an hour, due to destruction of a fibrin ferment. As a more persistent phenomenon there appear "vaso-tonins", vaso-pressor, or occasionally, depressor substances which have been much studied. The older literature is reviewed by Janeway, Richardson & Park (1918), and the reader is referred to their paper for a more detailed statement of the history of this subject. Outstanding in importance was the discovery of Stevens & Lee (1884) and Brodie (1903), confirmed and extended by O'Connor (1912), that the vaso-constrictor substance appears in connection with the process of clotting, and is absent from citrated plasma. The substance is definitely not adrenalin. While Battelli (1905) believed that his "vaso-constrictines" were destroyed by a temperature of 58° C., most other workers report the substance to be relatively thermostabile (Schlayer, 1907; Stewart & Harvey, 1912; Janeway, Richardson & Park, 1918; Meyer, 1925; Hashimoto, 1928). It is therefore not protein, but the chemical nature of the pressor material still remains in doubt. Friedberger & Seidenberg (1927) state that serum produced by coagulation at 0° C. has almost no vaso-constrictor effect. Stewart & Harvey (1912) described a vaso-dilator substance in both plasma and serum which is specific for the kidney, acting directly on muscle coats of arteries. It is a protein of the albumin class, and is precipitated by boiling and by alcohol. Freund (1920) distinguishes an earlier vaso-dilator, and a later vaso-constrictor phase. Confirming earlier observations by Zucker & Stewart (1913) and Le Sourd & Pagniez (1914), Freund stresses the importance of the blood platelets as a source of the material. Zipf (1931) has recently identified Freund's vaso-depressor substance as adenosine phosphoric acid and later gives a detailed analysis of its action (1932). Fiske (1934) has confirmed this finding. Both types of vaso-tonins are definitely not present in normal blood (Bodenstab, 1928). Freund, and later Bornstein (1926), described the disappearance of the vaso-tonins from serum in about 24 hours. Bayliss & Ogden (1933) distinguish "temporary" and "permanent" vaso-tonins. The "temporary" substance disappears after 48-72 hours, the "permanent" persists indefinitely. Goldberger (1931, 1932) also distinguishes a labile and a stable substance, the latter derived from disintegrating cells, and failing to appear if the cells are at once removed. Sapegno & Maestri (1931) find that insulin greatly increases the vaso-constrictor effect.

Recent studies suggest very strongly that at least part of the pressor effect is due simply to particulate material which mechanically blocks the smallest blood vessels. Eichholtz & Verney (1924) found that the vaso-tonins were inactivated by passing the defibrinated blood through the heart-lung preparation, and this procedure was adopted by Anrep and Häusler (1928), who also used fine linen filters. Bornstein & Roese (1929) find that the vaso-constrictor substance is also slowly removed by the perfused extremities of the dog, but the placenta does not remove them (Budelmann, 1929). Hemingway (1931) believed that mechanical shaking of the dog blood can produce the substances. Bayliss & Ogden (1933) could not remove the vaso-tonins by filtering through cambric (50-70 μ pores), but Daly & Thorpe (1933) report that they can be largely removed if the blood is passed through five layers of

cambric (50–100 μ pores), leaving the filters clogged with a gelatinous film. This mechanical explanation is, however, made somewhat difficult by the observation that the constrictor effect of serum is prevented by ergotoxin (Heymanns, Bouckaert & Moraes, 1932; Bayliss & Ogden, 1933) which also tends to constrict the vessels. Since this drug is known to block vaso-constrictor impulses over sympathetic fibers, it is possible that the active substance in serum acts upon these nerve endings.

It is beyond the scope of this review to consider the various pressor substances which are thought to be developed in blood serum during disease. For a recent review of this field the reader is referred to the article by Page (1935).

Many of the above studies on the vaso-tonins have been done on whole defibrinated blood. Blood serum, however, shows all of the phenomena, and this discussion has been included as a practical guide for those who wish to prepare plasma or serum for use as a substitute for whole blood.

It is now widely recognized that due to its organic and colloidal constituents blood plasma or serum, when properly prepared, may replace a very considerable fraction of the whole blood in the mammalian body, without disturbance of normal function. Richet & Brodin (1917) were able to get recovery in dogs after replacement of as much as 96 per cent. of the normal blood by horse serum. Even better results were found by Richet, Brodin & Saint-Girons (1917) and Richet (1919), when plasma was used instead of serum. Couvreur & Clement (1919), however, could not save dogs with serum after hemorrhage. Other excellent results after replacement of blood by plasma or serum have been observed by Guthrie & Pike (1907), Mann (1918), Foster & Whipple (1922), Rossius (1925), Kallius (1929), Weech, Goettsch & Reeves (1933), and others. The use of plasma instead of whole blood for transfusions has come into clinical practice, particularly to terminate uncontrollable bleeding. Filatov & Kartaševskij (1935) report successful plasma transfusions in seventy-two human cases. They use blood plasma of group AB (see Snyder, 1929, for terminology), which does not agglutinate the red cells of any other blood group, so that preliminary tests of the patients are not necessary. Injections of concentrated serum may give phenomena of shock (Achard, Levy & Gallais, 1932).

The final demonstration of the availability of plasma and serum as blood substitutes has been recently given by Carrel & Lindbergh (1935) and Lindbergh (1935) who, using a strictly aseptic technique, have succeeded in maintaining life in various isolated organs perfused with serum for as long as one month. All normal functions, including growth, may be maintained. This significant development leads us on to a fascinating new field of study, where physiological function may be delicately analysed under rigorously controlled and simplified conditions.

III. HEMOGLOBIN-RINGER AND HEMOLYSED BLOOD

Hemoglobin is the most important and unique constituent of whole blood. It is reasonable to expect, therefore, that, when all other organic constituents are removed, dissolved hemoglobin will be able to confer, upon an otherwise synthetic

solution, an ability to function effectively as a substitute for whole blood. This is indeed the case, but, strangely enough, the exact opposite was long believed to be true, and it will become evident, as we proceed, that a number of precautions must be observed in the preparation and use of such solutions.

Toxic properties of laked blood were first described by Naunyn (1868, 1873), and Francken (1870) who reported a quick intravascular clotting of the blood soon after the injection of as little as 2 c.c. of laked rabbit blood into rabbits, with immediate cardiac failure and asphyxiation. They were shortly confirmed by Bert (1870) and by Plosz & Györgyai (1874). Bert injected a solution of dog hemoglobin into a dog, with immediate fatal results, and concluded that "*l'existence de globules sanguins... paraît indispensable pour la conservation des propriétés vitales élémentaires*". Many later workers have also reported that intravenous infusions or perfusions with hemolysed blood are toxic, although some have observed that minimal quantities are tolerated (Ponfick, 1875; Hoffman, 1877; Kronecker & M'Guire, 1878; Sachssendahl, 1880; Silbermann, 1886; Heffter, 1892; Kobert, cited by Paldrock, 1896; Mioni, 1904; Battelli, 1905; Couvreur & Clement, 1919; Roger, 1919; Yamakami, 1920; Conti, 1921 (intraperitoneal injections); Osborne, 1930; Field, 1931; and others). Marked febrile reactions were seen by Sachssendahl, Silbermann and Yamakami.

Other workers have failed to observe such deleterious effects of laked blood. Injections of considerable quantities of distilled water were early made into various mammals (Hermann, 1859; Bernard, 1859; von Regeczy, 1885; Adami, 1885; Bayliss, 1920 *a*), with hemolysis of some of the cells and the appearance of hemoglobinemia and hemoglobinuria, but without fatal consequences. Even rabbits, which are particularly sensitive material, may withstand large injections of distilled water (Sapinoso, Berg & Jobling, 1926), although the red cell ghosts collect in the sinuses of liver and spleen, and fatal results ensue when large enough amounts are used.

Similar indications that hemolysed blood is not always toxic may be derived from such work as that of Ringer (1882, 1885), who, in his classic studies of salt balance, used a solution of dried beef blood in water, diluted with five parts of saline solution, to represent normal blood. With this solution he avoided the toxic effects of pure salt solutions upon the perfused frog heart. His attention was at that time fixed upon the phenomena of salt balance, but it seems clear that disintegrated red cells were not toxic to the heart in his experiments. A little later Yeo (1885) attempted to estimate the oxygen consumption of the frog heart by observing spectroscopically the reduction of dissolved oxy-hemoglobin in the surrounding solution. The hearts continued to beat normally. While Heffter (1892) claimed that neither laked blood nor dissolved hemoglobin could maintain the beat of the perfused frog heart, Albanese (1893) shortly came to opposite conclusions, stating that "*das Herz ebenso gut arbeiten kann, bei vollständig zerstörten rothen Blutkörperchen, als bei intakten*". Rusch (1898) first perfused the mammalian heart (cat) with laked blood and observed a strong beat for some time. This gradually weakened and the perfusion pressure had to be steadily raised to maintain the flow. He ascribed this effect to the accumulation of the stromata in the finer vessels.

Perfusion studies of other organs have been successfully made with hemolysed blood. Although Hoffman (1877) had failed in the attempt, Munk (1887, 1888) perfused the isolated dog kidney with laked dog blood and demonstrated urine secretion and the synthesis of hippuric acid from sodium benzoate and glycol added to the blood. He concluded that hemoglobin in solution is able to perform its normal respiratory role. Poliakoff (1904) and Huebner (1905) were able to maintain irritability in the frog hind-limb preparation perfused with laked beef blood. Bayliss (1920 *a*) could detect no toxic effects with hemolysed cat blood perfusions of the hind leg of the cat, the only result being a slight permanent rise in blood pressure. Even the resuspended stromata were innocuous. He concluded that hemoglobin in solution is able to maintain the normal tissue respiration of this mammalian preparation.

Injections of dissolved hemoglobin have been used for blood volume determinations in dogs, instead of the usual dyes (Lee & Whipple, 1921; Carrier, Lee & Whipple, 1922; and Lee, Carrier & Whipple, 1922), without any evidence of deleterious effect. Even into human beings considerable quantities of dissolved human hemoglobin have been injected without any injurious effects whatsoever (Sellards & Minot, 1916).

The apparently contradictory findings of these two groups of workers may be at least partly explained on the basis of the following literature.

(1) *Relative toxicity of hemoglobin and stromata.* Alexander Schmidt and his students were early able to show that toxicity of hemolysed blood may arise from substances carried in the stromata, and not from the released hemoglobin. Schmidt (1875) first separated these two components of the red cell and found that the stromata alone were responsible for accelerating the coagulation of blood which he had observed upon the addition of laked cells. A somewhat similar conclusion was reached by Silbermann (1886) who ascribed the toxic effect of hemoglobin to its power to destroy the leucocytes, setting free from them a fibrin ferment.

A number of later workers have concluded that the toxic effect of hemolysed blood may be entirely prevented if the stromata are removed (Wooldridge, 1886; Krüger, 1888; Levy, 1904; Schmidt, 1907; Barratt & Yorke, 1909, 1912, 1914; Foix & Salin, 1912, 1913; Sellards & Minot, 1916).

(2) *Toxic effects arising from improper ion balance.* Kronecker (1882) first suggested that the toxic effect of laked blood might arise from the high potassium content of the hemolysed cells. Working under his direction M'Guire showed that laked rabbit blood was toxic for the perfused frog heart, but that the toxicity disappeared if the potassium had previously been removed by dialysis. Continuing the work of Heffter, Albanese and Rusch, Göthlin (1901) had poor results when he perfused frog hearts with laked beef blood until he added calcium chloride, whereupon he secured a powerful beat. This observation led Langendorff (1903, 1905) to a more careful study. He found that laked cat blood could maintain the beat of the isolated cat heart, but at a subnormal level, which became normal upon the addition of calcium chloride. Similarly laked dog blood could maintain the dog heart but laked rabbit blood did not maintain the beat of the isolated rabbit heart. Suspecting

that the differing potassium content of the different cells, which had now been accurately determined by Abderhalden (1899), might be responsible for these diverse results he asked Brandenburg (1903) to make a systematic study with various laked mammalian bloods. It was found that bloods with cells containing high concentrations of potassium (human, pig, guinea-pig, rabbit, horse) were very toxic for the frog heart, that bloods with lower potassium contents (calf, sheep, goat) maintained the beat at a subnormal level, whereas the bloods of cats and dogs, very low in potassium, were not toxic, and gave a continuing beat for 5-6 hours. The addition of extra calcium to bloods with high cellular potassium partly antagonized their toxicity.

It will be noted that the first reports of the toxicity of laked blood were made with rabbits and it is highly probable that at least a part of the effect must be referred to the high potassium content of the red cells. The toxic effects observed by Mioni (1904) and Battelli (1905) in the rabbit and by Field (1931) in the guinea-pig may also be largely explained in the same way.

(3) *Anaphylactic reactions to repeated hemoglobin injections.* Heidelberger & Landsteiner (1923) have shown that hemoglobin is a weak antigen, hence a second injection of laked blood or dissolved hemoglobin may give anaphylactic shock. Moldovan & Isaicu (1926) attribute the effects observed to the antigenic action of the stromata of the red cells, but all other more recent observers agree that hemoglobin itself is the responsible agent (Engelhardt, 1925; Hektoen & Schulhof, 1927; Gussew, 1929; and Yasui, 1929).

(4) *Vaso-constrictor action of dissolved hemoglobin.* Various workers have described a vaso-constrictor effect of injections of dissolved hemoglobin. Rusch (1898), Bayliss (1920 *a*), and Phemister & Handy (1927) used laked whole blood, and the vaso-constrictions observed by them may have been due to serum components discussed previously in this paper. Hirschfeld & Modrakowski (1911), Brocking & Trendelenberg (1911), and Kaufman (1913) observed vaso-constrictor effects upon the injection of washed and laked corpuscles, but blood platelets may have been responsible. More recently Reid (1929) and Mason & Mann (1931) have described a vaso-constrictor effect localized in the kidney upon the injection into dogs of small amounts of distilled water (5 c.c.) or of purified hemoglobin solution, whereas stroma suspension had no effect. Osborne (1930) observed an immediate fall in blood pressure in dogs upon the injection of small amounts of hemolysed dog-blood whereas Field (1931) observed an initial sharp rise of blood pressure in guinea-pigs, with later fall; lethal effects occurred for both investigators. Amberson *et al.* (1934) found that massive injections of dissolved beef hemoglobin into cats and dogs produced an initial moderate vaso-dilatation, with a later and sustained vaso-constrictor effect.

We have recently examined the effect of the injection of small quantities of dissolved hemoglobin, obtained by hemolyzing various mammalian red cells after these have been washed free of serum. After hemolysis by adding distilled water, the stromata were removed by the centrifuge and sufficient sodium chloride added to render the solutions isotonic with mammalian blood. We have found that injections

of small amounts of such solutions (1-2 c.c.) always raise the blood pressure, apparently by exerting a vaso-constrictor effect. The solution made from rabbit cells has a much more powerful effect than that given by the solutions made from cat, dog, and beef cells, possibly because of the large amount of potassium originally present within the rabbit corpuscles, in part combined with the hemoglobin itself. In these experiments the presence of stromata was then tested, but the effect upon blood pressure was the same as in their absence.

Recently the ability of dissolved hemoglobin to function within the mammalian body has received renewed attention. Presuming that it is possible to avoid toxic effects by the proper choice of blood, and removal of the stromata, hemoglobin in solution presents many advantages for use as a constituent of a blood substitute, as follows.

(1) Hemoglobin is highly soluble in physiological salt solutions (Green, 1932).

(2) Dissolved hemoglobin takes up oxygen at a much faster rate than when it is within the red cells (Hartridge & Roughton, 1927).

(3) The hemoglobin molecule is approximately equal in size to that of serum albumin. Its molecular weight is 68,000 (Svedberg & Nichols, 1927; Northrop & Anson, 1929). It therefore has a colloidal osmotic pressure which, weight for weight, is considerably higher than that of the normal plasma proteins (Adair, 1925; Austin, Sunderman & Camack, 1926; Wells, Miller & Drake, 1935). When added to a Ringer-Locke solution in an amount equal to its concentration in blood (12-14 per cent. for cat and dog) it exerts a colloidal osmotic pressure of 50-60 mm. mercury, *i.e.* approximately double the normal value for the serum proteins. Hemoglobin passes fairly readily into the lymph and may be detected in the cervical lymph duct of the dog 30-45 min. after injection (Haynes, 1932).

(4) Dissolved hemoglobin stimulates the production of new red corpuscles, either by some direct action on the hemopoietic tissues, or because it furnishes the necessary material for such production, or both. With the exception of Metis (1924), all observers are agreed as to this effect (Itami, 1909, 1910; Hess & Saxl, 1912; Hooper, Robscheit & Whipple, 1920; Furukawa, 1922; Naswitis, 1922; Zih, 1930; Ferrari, 1932 *b*). Injections of dissolved hemoglobin greatly increase the iron stored in spleen, bone marrow and liver (Bogniard & Whipple, 1932).

Influenced by such considerations Salaskin and Kriwsky (1931) added dissolved hemoglobin to Ringer-Locke solution for the perfusion of the mammalian liver, and were able to demonstrate the synthesis of urea from ammonium carbonate, although pure Ringer-Locke was ineffective. Amberson & Höber (1932) and McClanahan & Amberson (1935) used hemoglobin-Ringer-Locke solutions for the perfusion of isolated salivary glands. With a solution containing 5 per cent. hemoglobin we were able to prevent the edema associated with perfusion by pure Ringer-Locke, and to delay the onset of the vaso-constriction produced by defibrinated blood or serum. We were led to recognize that hemoglobin in solution can perform the dual role of maintaining the colloidal osmotic pressure and of transporting oxygen in the mammalian preparations, just as it has been recognized to act where present in solution in the blood of invertebrate animals (Redfield, 1933).

These observations on isolated perfused tissues led us to attempt the replacement of all or most of the normal blood in cats, dogs, and rabbits by hemoglobin-Ringer solutions. We were able to demonstrate (Amberson, Flexner, Steggerda, Mulder *et al.* 1933, 1934) that these animals show an essentially normal behavior for many hours after the practically complete replacement of their normal blood by hemoglobin-Ringer solutions containing 12-14 per cent. hemoglobin. They recover consciousness and are able to walk and run. We found that the blood pressure is well maintained, and that the oxygen consumption of the whole animal and of the heart-lung preparation continues unaltered when hemoglobin-Ringer is substituted for normal blood (Mulder, Amberson, Steggerda & Flexner, 1934). In the absence of red corpuscles the hemoglobin in solution gradually changes over into methemoglobin, and the hemoglobin disappears from the blood stream, passing into urine and lymph, and being taken up by cells of the reticulo-endothelial system. As long as the hemoglobin concentration remains high the animals exhibit an essentially normal behavior. They die of asphyxia when the hemoglobin is reduced to 3 per cent.

In the development of these experiments with hemoglobin-Ringer we were originally much influenced by the claim of Barcroft (1922) that hemoglobin in solution would produce so high a viscosity and colloidal osmotic pressure that it would cause derangement of function. The experiments clearly show that, even when hemoglobin is present in solution in the concentration found in normal blood, there is no such effect as Barcroft expected. The viscosity of a 14 per cent. hemoglobin-Ringer is indeed somewhat higher than that of normal blood, and its colloidal osmotic pressure is approximately double the normal, but the fairly ready passage of hemoglobin into the tissue fluids and lymph appears to give an oncotic pressure approximating the normal closely enough to prevent any marked upset of the water balance. We fully agree, however, with Barcroft's argument that the red cell exists to prevent escape of hemoglobin into urine and lymph and to furnish a chemical environment in which hemoglobin may function with maximal efficiency, to which we would add the fact that a major factor in this chemical control is to maintain hemoglobin in its reduced state, electrochemically speaking, so that it is not transformed into physiologically useless methemoglobin.

In these experiments we have seen the kidney damage which has been stressed by so many other students who have made massive injections of hemoglobin (Schmidt, 1907; Yorke, 1911; and others). With dissolved hemoglobin in such concentration the kidney threshold is greatly exceeded (Pearce, Austin & Eisenbrey, 1912; Sellards & Minot, 1916; Manwell & Whipple, 1929; Lichty, Havill & Whipple, 1932; Havill, Lichty, Taylor & Whipple, 1932; Havill, Lichty & Whipple, 1932; Newman & Whipple, 1932; Bayliss, Kerridge & Russell, 1933). Hemoglobin collects in the form of granules or crystals in capsules and tubules, the whole kidney swells, and becomes almost black in color. From it, at autopsy, may be expressed a dark red fluid rich in methemoglobin. Yet these conditions are apparently not in themselves lethal or irreversible. We have frequently replaced as much as 80 per cent. of the normal blood with hemoglobin-Ringer, and had indefinite survivals of our animals, enough red cells being left to prevent asphyxia.

Within a week, in cats, the plasma is clear of hemoglobin, the kidney pathology has vanished, and the red cells are well on the way to recovery. By observing urine and the intraocular fluid in animals which survive, we have concluded that massive injections of dissolved hemoglobin must leave the blood stream in 48-72 hours. Smaller amounts are even more quickly eliminated (Barratt & Yorke, 1909; Pearce, Austin & Eisenbrey, 1912; Drabkin, Widerman & Landow, 1935).

Höber and Meirowsky (1932) and Webster, Engel, Laug & Amberson (1934) have used hemoglobin-Ringer for the perfusion of isolated frog kidneys. When the hemoglobin is more than 1 per cent. urine will not form. Below that value the hemoglobin comes through into the urine, and much more appears when the perfusion solutions are slightly acid than when they are alkaline.

Carrel & Lindbergh (1935), whose work is more fully discussed in the preceding section, have recently added dissolved hemoglobin to some of their sera for perfusion of excised tissues.

Brown & Dale (1936) have just reported favorable results with hemoglobin-Ringer in the perfusion of the hind limbs of the cat, where normal vascular reactions to histamine, acetyl-choline, and other drugs persist for many hours, without appreciable edema. They remove the stromata with a Sharples supercentrifuge, and then sterilize by passing the fluid through a Seitz bacterial filter. They find that such a solution can be stored, without notable deterioration, for many weeks, at a temperature just below 0° C.

The claim of dissolved hemoglobin upon the attention of workers in this field is surely strengthened by the fact that it is normally so present in the blood of many invertebrate animals. With the exception of the blood pigment of *Chironomus*, these dissolved hemoglobins (erythrocruorins) have molecular weights of the order of 1,300,000 to 5,000,000 (Svedberg, 1933), whereas hemoglobins developed within corpuscles, whether vertebrate or invertebrate, have very much lower weights, ranging from 25,000 to 68,000. Invertebrate hemocyanins, also present in solution, similarly have a large size. Living organisms have arranged to prevent the escape of their respiratory proteins from the blood stream either by developing materials of high molecular weight, or by enclosing the hemoglobins within cells whose membranes are impermeable to them. Bayliss, Kerridge & Russell (1933) found that *Helix* hemocyanin is not excreted by the mammalian kidney. The behavior of the large-moleculed invertebrate hemoglobins in the mammalian body deserves study; we are planning a series of such experiments in the near future.

The further chemical transformations of injected hemoglobin are beyond the scope of this review but, from many studies designed to follow the production of bile pigments from dissolved hemoglobin injected into the blood stream, it is evident that frequently no toxic effects arise, even when the stromata are not removed (Stadelmann, 1882; Schurig, 1898; Whipple & Hooper, 1913; McNee & Prusik, 1924). The reader is referred to the reviews of Rich (1925) and of Barron (1931) for a discussion of this aspect of the problem, and for further citations of literature dealing with hemoglobin injections.

IV. GUM-SALINE

We may now turn to consider the use, in a blood substitute, of colloidal constituents not normally found in the blood. Of these gum acacia (arabic) has received the most attention, and has achieved the greatest measure of success. First used by Carl Ludwig (1863) in kidney perfusion studies gum acacia has attained a steadily increasing importance in physiological work, and from the time of its first clinical application by Hurwitz (1917) it has grown in favor as a therapeutic agent.

Chemical constitution of gum acacia. Although O'Sullivan (1884, 1889) had early recognized the presence in gum acacia of both a pentose, arabinose, and a hexose sugar, galactose, and had assigned the formula $2 (C_{10}H_{16}O_8) \cdot 4 (C_{12}H_{20}O_{10}) \cdot (C_{23}H_{30}O_{18})$, calling it "diarabinan-tetra-galactanarabic acid", the early physiologists generally speak of gum acacia as a pentosan, and the furfural test has been widely used for its quantitative estimation. In the last six years the chemical and colloidal properties of gum acacia have received attention in a number of laboratories and we now have a fairly clear picture as to its constitution and behavior.

Butler & Cretcher (1929), Norman (1929), Heidelberger & Kendall (1929), and Weinmann (1929), independently and almost simultaneously came to the conclusion that the basic structure of gum acacia is a galactoso-glucuronic acid (an "aldobionic acid", of which both components are dextro-rotatory, with the hexose linked to the acid through the aldehyde group of the latter). Butler and Cretcher conclude that the gum molecule is made up of one molecule of this aldobionic acid, two molecules of galactose, three of arabinose, and one of rhamnose. Norman could not detect the presence of rhamnose. He reports, in one sample, after complete hydrolysis, 29.89 per cent. arabinose and 67.92 per cent. galactose. Heidelberger & Kendall have prepared the aldobionic acid in crystalline form. Challinor, Haworth & Hirst (1931) believe that the acid is structurally related to disaccharides and conclude that it is probably gentiobiouronic acid.

The crude gum consists mainly of the calcium, potassium and magnesium salts of arabic acid. By appropriate chemical treatment these cations may be largely replaced by sodium (Bayliss, 1916 *b*) or largely removed by electrodialysis (Schulz, 1924) so that a pure arabic acid is obtained which is nearly ash-free. Carrington, Haworth & Hirst (1934) have secured yields of pure *L*-arabinose up to 18 gm. per 100 gm. of purified arabic acid. According to Krantz (1929) and Brintzinger & Beier (1933, 1934) the crude gum is an effective buffer. The mid-point of its range is about pH 4.1-4.2. Unless well buffered by other salts, therefore, gum solutions tend to go acid. The need for such buffering was stressed by Atzler (1923) and Lehmann (1923), who added 1.2 gm. sodium bicarbonate per liter to a 7 per cent. gum-Locke solution to get a pH of 7.3-7.5.

A close chemical relationship exists between gum acacia and the polysaccharides produced by some bacteria. Hopkins, Peterson & Fred (1931) have identified glucoso-glucuronic acid as a constituent of the gum produced by root nodule bacteria, and there is a distinct possibility that gum acacia itself is produced by bacterial action. Heidelberger, Avery & Goebel (1929) have shown that the aldobionic

acid derived from gum acacia is comparable in its precipitating activity for Type II and Type III antipneumococcus sera with the bacterial "soluble specific substances" themselves. These latter substances, discovered by Dochez & Avery (1917) were later found to be polysaccharides (Heidelberger & Avery, 1923) and from them an aldobionic acid may be isolated which appears to be glucoso-glucuronic acid (Heidelberger & Goebel, 1927; Goebel, 1927). It is isomeric with the galactoso-glucuronic acid of gum arabic.

Molecular weight and aggregation state. If we accept the composition for gum arabic suggested by Butler and Cretcher we arrive at a molecular weight of about 1500. This agrees well with cryoscopic determinations in dilute solutions. Burton (1921) arrived at the value of 1800 from such measurements, and at 1750 by Euler's friction law. Tiebackx (1922) arrived at values of 1800-2400 by the freezing-point method. Amy (1929) gives 1600 by alkaline titration of the pure acid. 6.5 c.c. of 0.1 *N* alkali are required to convert 1 gm. of acid to the salt. Thomas & Murray (1928) find that 8.5 c.c. of 0.1 *N* alkali combine with 1 g. of arabic acid, and calculate the molecular weight to be about 1200. Amy gives a dissociation constant of 2×10^{-4} , but Briggs (1934) finds that arabic acid does not act as a simple monobasic acid, but rather as a mixture of acids with varying ionization constants, and that the value of the "constant" decreases rapidly with increased dilution from a value of about $K = 1 \times 10^{-3}$ for a concentration of 50-100 gm. of acid per 1000 gm. of water to near $K = 2 \times 10^{-7}$ at infinite dilution. "It is assumed that reversible peptization of the micella occurs when the solution is diluted and that this is accompanied by a marked decrease in the dissociation tendency of the acid groups present on the micella."

If gum acacia could exist only in molecular solution it could hardly serve any useful purpose in physiology, even when highly hydrated, since its molecule would be too small to remain long within the blood stream. In more concentrated solution, however, it behaves as a typical lyophilic colloid, and must become highly aggregated. Riddell & Davies (1931) have described anomalous adsorption curves for gum acacia on lithographic stone and charcoal, showing that while adsorption is rapid at low concentrations it is actually very much less from a 6 per cent. than from a 1 per cent. gum solution. They ascribe this anomalous effect to "a change in the molecular condition of gum arabic", so that the gum "exists as a simple electrolyte below 0.5 per cent., but aggregates to form a colloid above 2 per cent.", with micelles beginning to appear between 0.5 and 1.0 per cent. The viscosity of gum solutions is markedly influenced by the *pH* and depressed by salts (Spencer & Drummond, 1927; Thomas & Murray, 1928; Krut & Tendeloo, 1929), probably as a result of the dehydration of the sol. From these and other physico-chemical studies Thomas & Murray conclude that "gum arabic exists in the form of micelles like gelatine". Gum solutions change their viscosity greatly as the temperature is changed, suggesting considerable variations in their aggregation state. Went (1928) found a rise in viscosity as the temperature was increased from 15 to 36°C., but at higher temperatures it falls again (Taft & Malm, 1931; and Boutaric & Roy, 1934). Gum solutions increase in viscosity more rapidly than their

concentration is increased (Trommsdorff, 1901; Spencer & Drummond, 1927; Taft & Malm, 1931). This is a familiar characteristic of lyophilic colloids, suggesting an aggregation into micelles in the high concentration ranges. However, Taft & Malm (1931), disagreeing with all other recent students, finally conclude that "gum arabic is a strong electrolyte of high equivalent weight rather than existing as a colloidal phase when in contact with water". Kruyt & Tendeloo (1929) stress the importance of the electrokinetic potential in maintaining the colloidal system of gum acacia. Ostwald (1934) finds that gum solutions show the phenomenon of "structural viscosity" if measured at low pressures and temperature, which proves these sols to be true lyophilic colloids.

Colloidal osmotic pressure and viscosity. After earlier attempts at such measurements by Dutrochet, Funke and others, Pfeffer (1877), using copper ferrocyanide precipitation membranes, secured the first dependable values. In Table I we have

Table I. *Measurements of the colloidal osmotic pressure of gum-acacia solutions*

Concentration of gum, %	Salt content of medium	pH	Colloidal osmotic pressure mm. Hg.	Author
1	o	—	65-72	Pfeffer, 1877
6	o	—	246-275	"
18	o	—	1180-1200	"
6	o	—	134	Moore & Roaf, 1907
1	0.9 % NaCl	—	12	Knowlton, 1911
7	o	—	218	Bayliss, 1916b
7	Locke	—	39	"
7 (Na salt)	Locke + NaHCO ₃ and 40 mm. CO ₂	7.4	29	Gasser, Erlanger & Meek, 1919
6 (Na salt)	o	CO ₂ free	274	"
6 (Na salt)	o	CO ₂ sat.	83	"
3	0.9 % NaCl	—	5.4	Krogh & Nakazawa, 1927
6	o	—	16	"
3	0.65 % NaCl	—	5.6	Drinker, 1927
6	"	—	18.6	"
1 (Arabic acid)	o	3.37	24	Thomas & Murray, 1928
1	o	6.86	16	"
3	Locke, slightly modified	7.3	16	Went, 1929
6	o	7.3	57.5	"
7 (Na salt)	0.2 % NaCl	—	96	Briggs, 1933
11 (Ca salt)	0.25 % CaCl ₂	—	96	"
6	o	4.5	56-58	Dodds & Haines, 1934
6	0.9 % NaCl	4.2	8.8	"
6	"	—	11	Onozaki & Sanada, 1935
12	"	—	29	"

brought his data together with readings secured more recently by various other investigators. It will be seen that there is little agreement among the values, and it would appear that we do not possess accurate information concerning this point, certainly under physiological conditions. From these readings we must recognize that gum acacia sols are very sensitive to a number of factors and that their aggregation state and colloidal osmotic pressures are similarly influenced, as follows:

- (1) Different samples vary considerably from each other.

(2) Variations in temperature have practically no influence on the colloidal osmotic pressure (Pfeffer, 1877; Krogh & Nakazawa, 1927).

(3) In common with the blood proteins (Krogh & Nakazawa, 1927) the colloidal osmotic pressure is depressed as the pH is lowered (Gasser, Erlanger & Meek, 1919; Briggs, 1933). Purified arabic acid, however, shows an increase to a maximum at pH 4.25, below which the value falls again (Thomas & Murray, 1928).

(4) The sodium salt gives higher values than the calcium salt (Bayliss, 1916 *b*; Gasser, Erlanger & Meek, 1919).

(5) The colloidal osmotic pressure is at its maximum in the absence of salt, and is greatly depressed as the salt concentration is raised (Moore & Roaf, 1907; Knowlton, 1911; Bayliss, 1916 *b*; Thomas & Murray, 1928; Went, 1929; Briggs, 1933; Dodds & Haines, 1934).

(6) In the presence of physiological salt concentrations, the colloidal osmotic pressure rises faster than the concentration (Krogh & Nakazawa, 1927; Drinker, 1927), as does that of the blood proteins (Verney, 1926; Kylin, 1934), but is proportional to the concentration in the absence of salt (Dodds & Haines, 1934).

Failure to control these and other factors is undoubtedly responsible for the wide variations in the values of Table I. If we accept 1500 as the molecular weight of gum acacia we would expect a 6 per cent. true molecular solution to have an osmotic pressure of about 700 mm. mercury. Actually the recorded values run from 9 to 60 mm. mercury, under conditions which we may believe approximate normal mammalian bodily conditions. These values indicate a particle size ranging from 17,000 to 120,000, and roughly accord with the view that a gum solution of this strength approximates the colloidal osmotic pressure of the normal plasma proteins. Further studies are needed. It must be recognized that injected gum acacia may influence the normal blood proteins, since Onozaki & Sanada (1935) have described a rise in the value of the colloidal osmotic pressure of the blood far greater than could be accounted for on the basis of a simple mixture of gum acacia with plasma.

The viscosity of gum-saline solutions is probably of little importance in their physiological use. Bayliss (1920 *b*) concluded that "the viscosity of an artificial fluid is unimportant, while the fact of its colloids having as high an osmotic pressure as those of the blood is the vital question". Viscosities under conditions approximating the physiological state have been measured by Trommsdorff (1901), Bayliss (1916 *b*), Zondek (1921) and Went (1928). The first three found a 3 per cent. gum-saline to be isoviscous with normal serum. Bayliss and Zondek considered a 6 per cent. solution to be approximately isoviscous with whole mammalian blood. Trommsdorff, however, gives 9-10 per cent. as the correct value. More recently Went has made a careful study and gives the following values:

	Viscosity at 20° C. (water = 1)
Whole blood (50 % cells)	4.2
Blood serum	1.9
6 % gum-Ringer (unpurified gum)	3.0
6 % gum-Ringer (purified gum)	2.1
6 % gum-Ringer (purified) + 50 % cells	4.4

Influence of gum acacia on the physico-chemical properties of blood. Gum acacia enormously hastens the sedimentation of the red cells, particularly if the normal plasma proteins are absent. Bayliss (1916 b) first observed this effect, but believed that he was dealing only with a pseudo-agglutination which would not appear in normally circulating blood. This action of gum has, however, been urged against its use by some of its critics, and has been particularly emphasized by Kruse (1919, 1920), Hanzlik & Karsner (1920), Linzenmeier (1920), Appleman (1925), Schwartze, Cretcher & Hann (1928) and Lucia & Brown (1934). Bacterial agglutination is also facilitated by gum (Moretti & Aragona, 1928). Lucia & Brown have carried out *in vitro* studies of the effect of gum acacia upon the suspension stability of red cells in the absence of protein. Maximum instability is at 3-4 per cent. gum, with diminishing effects at higher and lower concentrations.

In our work we have encountered this effect in every experiment where red cells are added to gum saline. In several of our earlier experiments we encountered partial paralysis in our animals during recovery from total plasmapheresis. Suspecting that masses of agglutinated cells might be forming emboli in the brain and cord, we changed our method so as to add the red cells to the gum just before the injection. With this technique we have had no further trouble, and feel sure that agglutination cannot occur *in vivo* in circulating blood.

A more serious influence of gum-saline is the effect on the oxygen capacity of the blood recently reported by Christie, Phatak & Olney (1935). According to them gum markedly reduces the oxygen content of the blood, presumably by covering the surfaces of the red cells. The effect is sufficiently powerful (about 40 per cent.) to suggest that great care should be exercised in the use of gum when anemia is present, and possibly explains the war-time experience that gum-saline rarely saves human patients if the red cells are less than 25 per cent.

There are other indications that gum coats the surfaces of the formed elements of the blood. Kodera (1928) observed that gum inhibits the destruction of acetylcholine by red blood cells, and concluded that the gum surrounds them. In making white cell counts Walker (1932) found it difficult to hemolyze red cells in the presence of gum with the usual agents, and advised the use of 0.1 N HCl instead. The agglutinating influence of gum must be intimately connected with this surface adsorption.

According to Takeda (1929) gum-saline infusions increase the coagulability of the blood, and this may be partly because of the high calcium content (Walker, 1931), but since a similar effect is observed with almost all infusions (Van der Velden, 1909; Barach, Mason & Jones, 1922) this action is not of much significance.

Gum-saline in perfusion studies of isolated organs. (1) *Kidney.* The first recorded use of gum-saline in physiological work is by Carl Ludwig (1863)¹ who observed urine formation and absence of edema in a pig kidney perfused with a 3 per cent. gum solution. Upon clamping the vein Ludwig observed a cessation of urine flow. Tammann (1896) repeated this experiment using gum-saline under a pressure of

¹ I have been unable to consult this paper directly. It is cited by Tammann (1896) who quotes extensively from it.

100 mm. mercury. He secured a different result, observing a great increase in urine formation after clamping the vein, the urine having a viscosity equal to that of the gum-saline itself, indicating a ready passage of gum into the urine under these conditions. Fujitani (1907), however, concluded that little gum passed into the urine of the rabbit kidney if there were a free perfusion flow, and Raposo (1932) reported, on the same preparation, an absence of edema if 3 per cent. gum-Locke was used, indicating that the capillary walls are not readily permeable for gum.

(2) *Hind-leg preparations.* Poliakoff (1904) observed a favorable influence of gum-saline in restoring irritability to the perfused frog leg, although it diminished the excitability of the fresh preparation. On the same preparation L  wen (1904) observed that adrenalin produced vaso-constriction when 1 per cent. gum-saline was used. Atzler & Lehmann (1921) perfused frog legs with 3½ per cent. gum-saline, buffered to various pH values, to study the effect of pH upon capillary size, and found a vaso-constriction in both acid and alkaline solutions, but later (1922) concluded that their solutions had not been adequately buffered. Drinker (1927) found increased capillary permeability, dilatation, and edema when the frog leg was perfused with a buffered 3 per cent. gum-saline. Upon the addition of horse serum in amounts greater than 15 per cent. the dilatation and edema disappeared. Drinker concluded that serum is necessary for the maintenance of normal capillary tone, in agreement with Krogh (see his summary in 1929), who, with Rehberg, had previously had excellent results with 3 per cent. gum-saline in their perfusion studies of the influence of pituitrine upon the tone of the capillaries in the frog's web.

Bayliss (1916 b) appears to have been the first to use gum-saline in mammalian hind-limb perfusions. He observed a normal vaso-dilatation in the hind leg of the cat, perfused with 6 per cent. gum-saline, when the arterial pressure was raised by injecting gum solutions into the jugular. (The leg remained in connection with the spinal cord through its nerves.) Dale & Richards (1918) successfully perfused the hind legs of cats with gum-Locke solutions and were able to demonstrate the vaso-dilator action of histamine if the perfusion fluid also contained both red blood corpuscles and a small amount of adrenalin. Burn & Dale (1926) later succeeded in getting histamine dilator effects upon the addition of adrenalin alone.

(3) *Intestine and pancreas.* Kestner (1919) reported that he had successfully perfused the small intestine and pancreas of dogs with 3 per cent. gum-saline and had observed no edema.

(4) *Frog heart.* The first recorded use of gum-saline in heart work is that of Bowditch (1871). He did not perfuse the heart, but merely placed a 4 per cent. gum-saline (0.5 per cent. sodium chloride) within the chamber of the isolated frog ventricle, attached directly to a recording manometer. He found an absence of "Treppe" with gum-saline, and other minor differences, but concluded that such a solution gave results essentially similar to those observed when serum was used. Heffter (1892), finding that the frog heart did not survive well when perfused with egg-albumin-saline, tested the effects of solutions of red cells suspended in 2 per cent. gum-saline (0.6 per cent. sodium chloride), carefully neutralized with sodium bicarbonate. He found that "die Gummil  sung scheint sogar ein besseres Medium

als die Eiweisslösung zu sein da in allen Versuchen die absolute Kraft unverändert geblieben ist". He concluded that the serum proteins are of no importance, so long as blood corpuscles are present, and the perfusion fluid approximates the physical properties of normal blood.

Shortly thereafter Albanese (1893) reported that he had successfully maintained the beat of the perfused frog heart with 2 per cent. gum-saline solutions saturated with oxygen and containing no blood elements whatsoever. Öhrn (1894) confirmed these findings and showed that such gum solutions could restore the heart after long perfusion with isotonic sodium chloride had suppressed the beat. Jacobj (1900) found hearts to beat for 3-4 hours, and to show the characteristic effect of digitalis.

The interpretation of these experimental results was attacked by Howell & Cooke (1893), Locke (1895), White (1896) and, much later, by Zondek (1921) and Meyer (1922). Howell & Cooke, confirming the claim that gum-saline solutions will maintain the heart beat, pointed out that the gum brings in sufficient calcium and potassium ions to balance the solution, and attributed the beneficial effects to this fact. Locke took the same view, and White could not confirm Öhrn's claim that gum-saline would recover hearts completely fatigued by long sodium chloride solution perfusion, although for him colloid-free Ringer solution did give recovery. Zondek and Meyer arrived at similar conclusions. Junkmann (1924) however found a decidedly beneficial effect upon the heart beat of the frog when Ringer solution was replaced by a gum-saline in which the calcium content had been reduced to the Ringer level, and concluded that gum acacia acts in some other way than merely by balancing the solutions or increasing their viscosity. A similar conclusion was reached by Nakashima (1926).

(5) *Mammalian heart.* Ueki (1924) found gum solutions, even when purified by electrodialysis and adjusted to pH 7.5, to give very much shorter survivals of perfused cat hearts than did Tyrode's solution. Akiyama, Ishii, Kubo & Sasaki (1931) found that with gum-saline the heart of the dog heart-lung preparation stopped in a few minutes or went into fibrillation. These poor results were due to oxygen lack as has been shown by the significant observations of Ort, Power & Markowitz (1931) and Ort & Markowitz (1931) that, in the absence of red corpuscles, gum-saline is unable to maintain the mammalian heart, with much gum leaving the blood stream and penetrating into the tissue, whereas with cells present little or no gum leaves the vessels and the hearts will beat for some hours. It is known that the permeability of the capillary wall for proteins is similarly increased by oxygen lack (Landis, 1928).

While some confusion exists in this perfusion literature the weight of testimony is favorable, and we may believe that gum solutions, when sufficiently purified and properly buffered, and in which at least 20 per cent. of red cells are suspended, afford us an effective blood substitute for the perfusion of isolated tissues, even in mammalian preparations. Few investigators have, however, adopted it in such work, and it has rather tended to go out of use.

Infusion of gum-saline into the whole animal body. The first injection of gum-saline into the whole animal body was done by Moutard-Martin & Richet (1881). They

studied the effect of gum, injected into dogs intravenously, "en solution concentrée", upon the polyuria induced by previous sugar injection, and observed a partial or complete suppression of urine flow, even though blood pressure rose greatly.

In 1894 Czerny studied the effect of injecting gum acacia, gelatine, protein and blood serum into cats. His animals died when more than 4.6 gm. per kg. of gum were injected. He observed a high blood viscosity. Spiro (1898) and Fujitani (1907) concluded that gum-saline usually has a diuretic action, but Pugliese (1910) arrived at opposite conclusions. He and Roger & Garnier (1913) both observed great blood dilutions after gum-saline infusions which persisted for many hours, showing that the gum did not readily leave the blood stream and was able to hold water within it. Knowlton (1911) soon confirmed Pugliese with regard to the effect of gum upon the kidney. He found that either gum or gelatine-saline can inhibit the diuresis produced by normal saline injections, although they are largely ineffective upon the diuresis caused by sodium sulphate. Later work has tended to show that, against a background of pre-existing diuresis, gum-saline is usually anti-diuretic (Mattill, Mayer & Sauer, 1920; Cori, 1921; Nonnenbruch, 1922), but in the normal animal there is usually little or no effect (White & Erlanger, 1920; Walker & Keith, 1930). Cori, however, sometimes observed a mild diuretic effect in dogs.

Morawitz (1906) was the first to show that an animal, which would otherwise die after a large hemorrhage, will survive if the lost blood is replaced by gum-saline. He removed blood from dogs and replaced it with 3 per cent. gum-saline until the plasma proteins were reduced as low as 2 per cent. His animals survived and he was able to follow the regeneration of the plasma proteins, his major interest. After Morawitz many workers have testified to the ability of gum-saline to maintain life in various mammals after extensive blood loss or traumatic shock and to restore blood pressure and blood volume. This literature is too extensive to discuss in detail but for those who wish to read further the following list of papers is given: Bayliss (1916 *a, b*, 1917, 1918, 1920 *b*, 1922 *a, b*), Mann (1918, 1920), Rous & Wilson (1918), Brodin, Richet & Saint-Girons (1918), Erlanger & Gasser (1919), Kestner (1919), Gesell (1919, 1921), White & Erlanger (1920), Matill, Mayer & Sauer (1920), Smith & Mendel (1920), Cushny & Lambie (1921), Kulz (1921), Gesell & Moyle (1922), Gesell, Foote & Capp (1922), Gesell, Capp & Foote (1922), Barthélemy (1924), Tesauro (1924), Wüllenweber & Koch (1926), Bricker, Suponitzkaja & Tscharni (1926), Bald (1927), Bucci (1927), Bucci & Magliulo (1928), Chiappini (1930), Walker & Keith (1930), Sasaki (1931), Beard & Blalock (1932), Blalock, Beard & Thuss (1932), Adolph, Gerbasi & Lepore (1934), and Freeman (1935). Meek & Gasser (1918) and McQuarrie & Davis (1920) have used gum for the determination of blood volume.

A further word should be said about the experiments of Gesell, Capp & Foote (1922). These workers were able to show (as Schlomovitz, Ronzone & Schlomovitz, 1924, have also shown) that the oxygen consumption of dogs falls off when the blood volume is reduced by hemorrhage. It is completely restored and maintained at normal when the lost blood is replaced by gum-saline.

The ability of gum-saline to restore blood pressure is lost in decapitate preparations (Bayliss, 1918) and even in normal animals after massive infusions of gum-saline without previous hemorrhage (Marrassini, 1922). Such infusions are accommodated without rise of blood pressure but even a small hemorrhage then leads to a great fall in blood pressure which cannot be restored by a new infusion. Nervous factors are clearly concerned with the regulation of water balance, and the relaxation of vascular tone, however induced, must greatly increase the ease of movement of colloid out of the blood stream. The importance of the nervous factors has again been stressed by Schlossberg & Sawyer (1933) who find that, after total sympathectomy, the blood pressure recovers very poorly from hemorrhage.

Even in normal animals a small number of observers have failed to get beneficial effects with gum. Kruse (1919, 1920) failed to get a rise of blood pressure after injecting gum-solution without salt. Penfield (1919) attempted to simulate war conditions by bleeding animals to shock level, and holding the blood pressure at this level for such times that "their condition resembled that which must frequently exist in wounded men at the front who have sustained severe and continued loss of blood". He was unable to show that gum-saline or gum-glucose was more efficacious in saving life than was an isotonic solution of sodium chloride. Henderson & Haggard (1922) state that "although temporarily beneficial, mere restoration of blood volume, even by a fluid approximating the physical properties of plasma, such as acacia solution, does not considerably increase the probability of ultimate recovery". They concluded that it is the loss of red corpuscles which is the critical factor in hemorrhage. Nonnenbruch (1922) and Appleman (1925) reported that the red-cell count in rabbits returned to normal within 30 min. to 2 hours after replacement of one-fourth of the blood with 6 per cent. gum-saline, a behavior essentially similar to that with Ringer-Locke. They concluded that most of the gum leaves the blood stream almost at once, although Appleman found small amounts present in the circulation for many days. Tsurumaki & Kurozawa (1924) reported that for them rabbits died more quickly after gum infusions than when nothing was done. Brodin, Richet & Saint-Girons (1918), Richet (1919) and Kallius (1929) have had favorable results with gum-saline, but consider it to be distinctly less effective in maintaining blood volume than is blood serum.

Crile, Rowland & Wallace (1924) have also opposed the use of gum. They bled Belgian hares and followed the temperatures of liver and brain as the lost blood was replaced by gum-saline or by blood transfusion. They report that the "oxidative power" of the brain is much diminished by the gum, so that the temperature continues to decline, although prompt recovery follows the injection of blood.

In this connection it should be mentioned that Takahashi (1935) has recently claimed that even small injections of gum-saline somewhat diminish the blood volume and appear to drive some of the blood corpuscles out of the blood back into the blood cell depots.

Our own experience with gum acacia has persuaded us that, when carefully used, it is very effective indeed in maintaining blood pressure and blood volume.

Extending the work of Ort, Power & Markowitz (1931) we have recently (Stanbury, Warweg & Amberson, 1936) been able to do plasmapheresis in dogs and cats so radical that the normal blood proteins are reduced to traces of 0.1–0.2 per cent. This may be accomplished by alternate bleedings and injections of red-cell-gum-saline. We bleed from the carotid artery and inject backward through the same cannula under pressure. We pass large quantities of our solution through the body until practically all of the original blood has been removed. The quantities perfused are of the order of 300–400 c.c. per kg. We routinely use some twenty bleedings and injections, 100 c.c. at a time. The blood pressure is maintained practically at the original normal even when the plasma proteins have been almost completely removed. The whole secret of success is to use sufficient red corpuscles of the same species to prevent anoxemia. When we add 30 per cent. or more of washed corpuscles to our gum solutions the animals come through the operations beautifully, and we are able to study the regeneration of the plasma proteins from practically a zero level. They survive for months and are normal in every respect. We have never seen shock, and only occasionally have we seen a transient edema localized in the neck, and possibly arising from some operative obstruction. The plasma proteins return rapidly to the blood stream, rising to a value of 1.5–2 per cent. in the first 24 hours although the original normal is recovered only after 100 hours in cats and 200 hours in dogs. The proteins return rapidly enough in both animals to take the place of the acacia as it vanishes from the blood stream so that at no time do symptoms of edema or shock appear.

Gum-saline in clinical practice. In this review we are interested in the general biological problem of blood substitution, rather than in therapeutic applications. For the sake of completeness, however, we give the following condensed statement of clinical experience.

Gum-saline was first employed clinically by Hurwitz (1917) who, stimulated by Hogan's (1915) earlier use of gelatine-saline, successfully treated the first cases in the San Francisco clinics in the summer of 1916. At the same time Bayliss was busy with his animal experiments in London, and so aroused the interest of army medical men that he was invited to visit the Western Front early in 1917 to arrange for a clinical application there. Gum-saline came into extensive use at advance medical stations, and the Base Hygiene Laboratory of the B.E.F. at Boulogne was shortly producing 75 liters of sterile gum solutions daily (Telfer, 1919). Lack of proper precautions in the preparation or administration of the solution, however, caused some failures, and considerable opposition to further use developed. Favorable results in the field were reported by Fraser & Cowell (1917), Cannon, Fraser & Hooper (1917), Drummond & Taylor (1918), Robertson & Bock (1918), Keith (1919), Lee (1919), and Ohler (1920). There was general agreement that it was less effective than whole blood transfusion, and found its best application in cases of moderate shock without great hemorrhage. Bernheim (1919) and an editorial writer for the *Journal of the American Medical Association* (1922) strongly criticized the use of gum, while a few clinical reports of death following its administration appeared (Olivecrona, 1921; Lee, 1922).

In contrast to these early warnings a fairly extensive literature has now accumulated which testifies to clinical success with the solution. Farrar (1920, 1921) used gum-glucose solutions (6 per cent. gum acacia, 20 per cent dextrose, 0.9 per cent. sodium chloride) in over 400 obstetrical and surgical cases with "no bad results of any description". Coburn (1924) and Coburn & Ward (1925) report 1000 successful cases where 6 per cent. gum-saline was used without a single mortality. They introduced the procedure of slow injections during operation at the rate of 4 c.c. per minute, often supplemented with blood transfusion. Masson (1927), McIndoe (1929), and Adson & McIndoe (1929), have used the same technique with success, particularly during intracranial operations. Huffman (1929) reports successful results in 300 surgical and shock cases. Dieckmann (1931) has successfully used hypertonic glucose and acacia to combat the concentration of the blood observed in eclampsia. Hartmann & Senn (1932), Hartmann, Senn, Nelson & Perley (1933), and Barach & Boyd (1935) have introduced the use of gum in the treatment of edema and of lipoid nephrosis, although Dick, Warweg & Andersch (1935) find that the beneficial effect of a first injection is lost with repeated injections, as the proteins fall to subnormal levels, aggravating the edema. Good, Mugrage & Weiskittel (1934) report favorable results in 111 cases of surgical shock.

Gum acacia is now in almost daily use in hundreds of hospitals throughout the world, and next to blood plasma unquestionably constitutes the most successful substitute for whole blood which has so far been developed.

Fate of gum acacia in the mammalian body. There has been a long controversy as to whether gum acacia is a food. In 1818 Pearson published a short pamphlet recommending the use of mixtures of pure white starch and gum arabic as an emergency diet for travelers in the desert regions of Africa. He states that Hasselquist, in 1766, describes how "above one thousand persons, belonging to an Abyssinian caravan, were supported for two months by gum arabic alone". He refers to work of Magendie who fed dogs on gum arabic for up to a month, only to find that they could not survive, but points out that it is hardly fair to conclude, from such experiments on carnivorous animals, that human beings cannot utilize the gum.

In the next half-century a number of investigators carried out chemical and feeding experiments with gum acacia, designed to test its digestibility, and concluded almost unanimously that, with the exception of herbivorous animals, mammals cannot digest it or absorb it from the digestive tract. This old literature is reviewed by Voit (1874) who, with his students, arrived at a different conclusion and claimed that gum may be at least partly digested, since they recovered only a portion of the ingested material in the excrement. Until recently there has been little further interest in the matter, although there are occasional references to a nutritive function of gum (Öhrn, 1894; Erlanger & Gasser, 1919). Recently Voskressensky (1924) and Nakaschima (1929) have been unable to maintain mammals on a pure gum diet and Lecoq (1934) has described symptoms akin to polyneuritis, leading to death. Nakaschima believed that intravenously injected gum can be utilized as a food. Although Uffelmann (1882) could detect no effect upon the rate of digestion of

protein by artificial gastric juice, when gum acacia was added, Bentivoglio & Palesa (1931) and Spolverini (1932) have recently urged the addition of gum to milk for infant feeding. It is said to increase the dispersion of the milk colloids (acting as "Schuttkolloid"; see also Voigt (1931), and Pauli, Russer & Schneider (1934) for a similar action in inorganic systems) and produces a greater stability and regularity in digestion, a diminution of intestinal putrefaction, and an absence of constipation. It appears that gum acacia may be attacked and broken down by the intestinal bacteria, and protozoa may also digest it (Nakaschima, 1929).

Andersch & Gibson (1934) have recently established, beyond further doubt, that gum acacia cannot be readily metabolized by the animal body, after intravenous injections. They have observed a deposition of gum in the livers of experimental animals, and of one human case, where it may be detected histologically. To a less extent gum is deposited in the spleen. They were able to recover as much as 30 per cent. of the injected acacia in the liver after three months. Dick, Warweg & Andersch (1935) have just reported that repeated small injections of acacia lower the plasma proteins, presumably by blocking the liver cells which produce them. Keith, Power & Wakefield (1935) have been able to detect traces of acacia in human serum three years after its injection. They believe this to be due to the "slow re-entry of the acacia into the blood stream from deposits formed in various tissues".

As a result of fixation by the tissues, together with some, often relatively slight, loss into the urine, gum acacia disappears fairly rapidly from the blood stream. Data on the rate of disappearance are given by Meek & Gasser (1918) (rabbits), Huffman (1929) (man), and Peoples & Phatak (1935) (dogs). From the average curve drawn through these three fairly consistent sets of data we obtain the following values, which are probably approximately valid for all mammals:

Hours	Relative concentration in blood
0	100
12	71
24	61
48	43
72	25
96	10
120	2

It is therefore apparent that, however efficacious they may be immediately after injection, gum saline injections cannot function by themselves to maintain blood volume and blood pressure for much more than 48 hours.

Anaphylactic reactions to gum acacia. We have already seen that gum acacia is chemically related to the antigenic polysaccharides produced by pneumococcus and other bacteria. It is therefore important to inquire whether it can also serve as an antigen. Bayliss (1916*b*) believed that it had no such action, and was shortly confirmed by De Kruif (1919) who could observe "no effects of an anaphylactiform nature" after injection into guinea-pigs, although it is known that such symptoms frequently follow the infusion of kaolin, agar, Witte's peptone and similar sub-

stances. Almost immediately and on the same material, Hanzlik & Karsner (1920) came to opposite conclusions. They classed gum acacia with those agents which injure both circulatory and respiratory systems, producing anaphylactoid symptoms including pulmonary distention, congestion and hemorrhage, cardiac dilatation, and depression of peristalsis. Their denunciation of gum was so bitter that some workers were persuaded to discontinue its use. We can only say that such terrible pathology has not been seen by most later investigators, although Toyoshima (1927) has made similar observations in rabbits. We can only conclude that the gum acacia was not properly prepared.

Other workers have reported indications of a mild anaphylactic effect. Busquet & Vischniac (1929) think that a first injection of gum produces an "immunity" to later injections, as evidenced by the disappearance of a transient hypotension which follows the first injection. Marrassini (1923) had previously seen the same effect, but ascribed it to hydroxyl ions, since gum-saline solutions rendered acid did not show it. Nagashima (1925) claims that whereas normal rabbit serum is unable to digest gum, such serum acquires this property after a first injection.

More definite in character are the reports of Maytum & Magath (1932) who have described anaphylactic reactions to gum acacia in man and the guinea-pig, upon a second injection, and of Uhlenhuth & Remy (1933, 1934) who find antibody formation in the rabbit in response to gum injections. They claim that some animals possess natural antibodies against gum (horse, ass, and, less strongly, beef, pig and camel) but that man and the guinea-pig are devoid of such natural protection. In their second paper, they report that antibodies do not form against such carbohydrate substances as amygdalin, glucosamine and glucose, even after repeated injections, and they also fail to get antibody reactions to gum acacia after it has been rendered N-free by purification. They conclude that the antigenic action of gum is due to traces of protein. Such traces of protein are undoubtedly present even in relatively pure specimens of gum. Spielman & Baldwin (1933) report 0.5 per cent. protein in acacia powder, and have observed a case of bronchial asthma in man definitely caused by gum infusion, with sensitivity confirmed by skin tests. We find traces of protein still present in the carefully prepared ampoules of acacia in solution furnished by the Eli Lilly Company. We have never, however, observed any toxic or anaphylactoid effects with these solutions in animal experiments, and the literature of successful acacia usage, here reviewed, gives assurance that such reactions must indeed be rare and of small importance.

Rivers & Ward (1935) have just introduced gum acacia into use in the preparation of desiccated vaccines, stating that it is "a harmless non-antigenic substance that...protects the active agent during the process of drying and resuspension". The ability of gum to combine with toxins may, however, make it unsuitable for use in certain diseases. Rogers (1919) found it absolutely detrimental in the treatment of Asiatic cholera, and concluded that it combined with the toxins and held them in the body. Wada (1932) reported that it may prevent the action of capillary poisons, such as histamine, but this may be due to vaso-constriction induced by its contained calcium.

V. GELATINE-SALINE

Ringer (1885) found that he could obtain recoveries in the frog heart exhausted by long sodium chloride perfusion if he added gelatine to the solution, but the effect was not so powerful as that obtained by blood serum. Czerny (1894), among other colloids, injected gelatine intravenously into cats and found that they tolerate up to 4 gm. per kg., above which value they died. He reported a high blood viscosity after such injections which was later confirmed by Spiro (1898) and by d'Errico (1907), who found gelatine to have a strong lymphagogenic action, and by Bottazzi, d'Errico & Jappelli (1908) and Buglia (1910). Pugliese (1910), however, reported that injections of concentrated gelatine solutions drew water out of the tissues and diminished both lymph and urine flow, but that lower concentrations and especially those made up in hypertonic sodium chloride gave diuresis and increased lymph flow. An anti-diuretic influence of gelatine was also reported by Knowlton (1911).

Gelatine is either non-antigenic (Starin, 1918; Kahn & McNeil, 1918) or very weakly so (Adant, 1930; Bruynoghe & Vassiliadis, 1930); hence there would appear to be little objection to its use on the score of possible anaphylaxis. However febrile reactions and death have been described after intravenous or intramuscular injections by Steabben (1925) and Dadlez & Koskowski (1929), and Spatolisano (1927) has observed hyperglycemia.

Gelatine-saline, while superior to a colloid-free saline, in its ability to restore and maintain blood pressure, is apparently relatively ineffective. Gelatine undoubtedly leaves the blood stream with fair ease. Buglia (1910) reported it still present in the blood after 40 hours, but Clark (1918) found that it began to disappear immediately after injection, and continued to do so at a fairly constant rate, being completely eliminated in 6-24 hours. Nonnenbruch (1922) takes a more extreme view, and claims that it completely disappears in 2 hours. Isotonic gelatine-saline has been infused by Roger & Garnier (1913), Boycott (1914), Bogert, Underhill & Mendel (1916), Bayliss (1916 *a*), Mann (1918), and Roskam (1921), for all of whom the gelatine left the blood vessels fairly rapidly, although the restoration of the original blood volume was slower than after the infusion of Ringer solution.

Another objection to its use has been raised because of its tendency to hasten coagulation and produce intravascular clotting. Gelatine was used by the ancient Chinese physicians as an hemostatic agent (Miwa, 1902), and its ability to shorten coagulation time has been reported by Dastre & Floresco (1896), Schmerz & Wischo (1918), and Takeda (1929). Schmerz & Wischo ascribe the effects to the calcium of the gelatine, and state that calcium-free gelatine retards rather than hastens coagulation.

Hogan & Fischer (1912) suggested the use of alkaline gelatine-saline for infusion work, accepting Fischer's thesis that such conditions as shock are characterized by acidosis and a movement of water out of the blood into the tissues, where the colloids become hydrated. Hogan (1915) later put the idea to clinical test, and reported six cases of the successful use of gelatine-saline. This work was the first

attempt to use a colloid clinically for infusion work and deserves recognition on that account since it stimulated Hurwitz (1917) and others to experiment with gum.

In recent years gum saline has almost completely replaced gelatine-saline. The use of the latter can no longer be recommended.

VI. GENERAL CONCLUSIONS

We may now bring together the major conclusions to which our review leads. It will now be apparent that, for the mammalian body, it is impossible to prepare a substitute for whole blood unless we use some component of blood itself. Gum-saline is no exception since our blood substitute, after gum infusion, is really a mixture of whole blood and gum-saline, containing some red cells. There is no possible substitute for hemoglobin. Either contained within red cells or dissolved in solution it *must* be present in the final mixture within the body in a concentration greater than 3 per cent.

However adequate they may be in restoring blood volume after hemorrhage, neither blood plasma nor gum-saline can possibly replace all of the mammalian blood. If such solutions are infused as blood is withdrawn the animal invariably dies when the red cells have been reduced to about 10 per cent. Gum-saline may, however, replace *all* of the blood plasma, if we employ red-cell-gum-saline solutions, and the concentration of the plasma colloids, both protein and lipid, may be reduced to the vanishing point, at least for short periods, without evidence of shock or edema. Great experimental difficulties arise to prevent the continuation of this unusual condition for long, since infusions cannot be maintained indefinitely, and, with their cessation, the normal blood colloids are fairly rapidly replaced while the gum simultaneously leaves the blood stream. For several hours, however, the blood pressure and volume may be maintained by gum-saline in the nearly complete absence of protein and lipid. By this demonstration we are convinced that the major significance of the blood colloids is purely physical, in maintaining a colloidal osmotic pressure. The capillary dilatation and edema observed in perfusion studies by some workers must, we believe, be referred to oxygen lack.

The red cells themselves are not basically necessary, and may be practically all removed from the body, but hemoglobin in solution must then take their place. Only thus is mammalian life without red cells possible. Of all of the above discussed substitutes, hemoglobin Ringer-Locke permits the most radical departure from normal body conditions, with hemoglobin in solution cast in the dual role of carrying oxygen and simultaneously maintaining the colloidal osmotic pressure, a double function never found normally in any vertebrate animal, although present in those invertebrates which carry their hemoglobin in solution in their blood. Farther than this we cannot depart from normal blood and still have the whole mammalian body live. We conclude, as we began, with the recognition that there is no complete substitute for blood.

VII. SUMMARY

1. Solutions of crystalloids cannot be considered as substitutes for mammalian blood since they quickly leave the blood stream and are unable to maintain blood volume and pressure. This is true even of hypertonic solutions.

2. The only effective blood substitutes are those which contain sufficient colloidal material to give a colloidal osmotic pressure approximating that of normal blood. Blood plasma, blood serum, Ringer-Locke solutions containing dissolved hemoglobin, and gum-saline are the only substitutes of practical importance. Gelatine-saline has also been used, but it is not now recommended.

3. Blood plasma is an effective substitute for much of the blood if the red cells in the mixture in the body are not too much reduced. Investigators are not agreed as to which organic constituent of blood plasma is most important. One group of workers supports the thesis that the plasma proteins are of major importance, another that it is the plasma lipoids which are most significant. Nearly all are agreed in the recognition of some organic colloidal factor.

4. Blood plasma is to be preferred to serum because of the formation, in the latter, of vaso-dilator or constrictor substances, produced in the act of clotting. The vaso-motor effects are partly chemical, partly mechanical in origin. Carefully prepared serum may, however, be used.

5. The oxygen capacity of the blood substitute is of vital importance. There is no substitute for hemoglobin. It must be present within the blood in a concentration of 3 per cent. or more, either within red cells, or dissolved in solution. The final mixture within the body cannot drop below this value.

6. When carefully prepared, properly balanced as to ion content, and well buffered, gum-saline solutions furnish an effective blood substitute, particularly when washed red cells of the same species are suspended in the solutions. With solutions of the latter type it is possible to remove all of the normal blood and reduce the concentration of the normal plasma colloids to the vanishing point. In such total plasmapheresis there should be at least 30 per cent. of red cells added to the gum-saline.

7. Difficulties arise in the use of gum-saline for the following reasons:

(a) The sedimentation rate of the red cells is greatly increased.

(b) Gum appears to coat the red cells and considerably reduces their ability to combine with oxygen.

(c) Gum leaves the blood stream with fair ease and cannot maintain the colloidal osmotic pressure of the blood for much more than 48 hours.

(d) Gum is fixed in some of the tissues, particularly in the liver, and may be held in the body for as long as three years.

(e) In association with its retention in the liver it is said considerably to diminish the concentration of the plasma proteins, possibly by blocking the liver.

(f) It is chemically related to the antigenic polysaccharides produced by pneumococcus and other bacteria. Occasional instances of an antigenic action are on record, but these cases appear to be very rare.

8. In spite of these difficulties gum-saline has had an increasing use in experimental work and clinical practice, and is to be highly recommended for many purposes.

9. Dissolved hemoglobin is not toxic to the vertebrate body if it has been properly freed from stromata, and if the solution is properly balanced. It is able both to carry oxygen and maintain the colloidal osmotic pressure. With such hemoglobin-Ringer-Locke solutions it is possible to wash out all of the normal blood and get mammalian preparations practically devoid of all cellular components in the circulating fluid. Oxygen consumption continues at the normal level. Hemoglobin in solution, however, leaves the blood vessels rather quickly, passing into lymph and urine, and being taken up by cells of the reticulo-endothelial system. It is also slowly changed into methemoglobin. It is not able, therefore, to maintain life for more than 36 hours after complete removal of the normal blood.

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EVOLUTION AND ADAPTATION IN THE DIGESTIVE SYSTEM OF THE METAZOA

By C. M. YONGE

(University of Bristol)

(Received 29 January 1936)

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I. INTRODUCTION

IN a previous contribution to *Biological Reviews* (Yonge, 1928*a*), an account was given of feeding mechanisms in the invertebrates, while elsewhere (Yonge, 1931*b*) the nature of the digestive processes in marine invertebrates and fishes has been reviewed. The present article represents the expansion of a communication read before Section D of the British Association for the Advancement of Science in 1932, and in it attention is confined to the evolution and specialization of form and function in the gut of the Metazoa, excluding the vertebrates to which only passing references are made. Certain general conclusions have been reached as a result of extensive investigations into the comparative physiology of feeding and digestion in a variety of invertebrates, and these are incorporated in this article. The aim throughout has been to emphasize factors of general biological significance rather than details of morphology or physiology. No attempt has been made to provide

a complete list of references to the extensive and very scattered literature on this subject; only the more important papers are quoted which deal with aspects of the subject which are here especially stressed.

The original development of the power of holozoic nutrition was responsible for the appearance of the first animals. This could have taken place only if the cell membrane was of such a nature as to permit phagocytic ingestion. Within the food vacuoles in the protoplasm the capacity for digestion may have involved changes in the sucroclastic, proteoclastic and lipoclastic enzymes which were all presumably present in the simple plants from which the first animals probably evolved. The food of these first animals presumably consisted of fragments of organic matter derived from plants or else the entire bodies of these. In the course of evolution in both plant and animal kingdoms the available food became progressively more diversified—living plants and animals of every size, all or portions of which could be consumed, or in or on which a parasitic existence was possible, and also organic matter derived from the dead bodies of these. This evolution of food was accompanied, *pari passu*, by an evolution of the mechanisms of feeding and digestion, the latter involving changes both in the structure and functioning of the gut.

Evolution within the animal kingdom has taken two main paths: elaboration within a single cell membrane in the case of the Protozoa, and the formation of a multicellular organism with specialization of the constituent cells in the Metazoa. With the former this article is not concerned. Evolution there is related primarily to feeding mechanisms and to specialization of enzymes in correlation with the nature of the food, much important information on this subject and extensive references to literature being provided by Sandon (1932). Within the Metazoa, the Porifera have been evolved independently; they alone do not possess a true gut. The paragaster is no more than a cavity lined with choanocytes which ingest food particles in the same manner as the choanoflagellates (see van Trigt (1919) for the best account of feeding and digestion in the Porifera).

The remaining Metazoa all possess a true gut. The elaboration of this, and the morphological and physiological adaptations which have enabled different animals to exploit every possible source of food, form the subject-matter of this article.

II. PERSISTENCE OF INTRACELLULAR DIGESTION

In the primitive unicellular animals, digestion must have taken place within the cell after ingestion. This is true of the free-living Protozoa although there are cases where enzymes are extruded, *e.g.* in *Vampyrella*, most recently investigated by Lloyd (1926), where secreted enzymes digest the cellulose wall of *Spirogyra* on which the animal feeds. In the Porifera, digestion is exclusively intracellular. In the Enterozoa there is a general tendency for intracellular digestion to be first assisted by, and finally entirely replaced by, extracellular digestion. We may divide into two groups animals in which intracellular digestion persists: (1) those which are primitive in structure, and (2) those which are more highly evolved but have retained intracellular digestion to a greater or less extent in correlation with the type of food collected by the feeding mechanism.

(1) This includes the Coelenterata, Ctenophora, most Turbellaria and *Limulus*. In the Coelenterata (see Yonge, 1930*b*, 1931*a*, for most recent work and full references to literature) extracellular digestion is confined to the preliminary digestion of proteins only, digestion of carbohydrates, fats and the final conversion of proteins into amino acids are all carried out intracellularly. The same conditions apparently also exist in the Ctenophora. There is considerable controversy as to the exact conditions in the triclad Turbellaria. Certain workers, such as Arnold (1909) and Löhner (1916), came to the conclusion that intracellular digestion was preceded by some extracellular digestion; others, such as Westblad (1923) and Willier, Hyman & Rifenburgh (1925), failed to find any certain indication of extracellular digestion. Undoubtedly intracellular digestion is very highly developed in these animals (see also Kelley, 1931, and Schlottke, 1933*a*), but some preliminary digestion of protein, these animals being carnivorous like the coelenterates, appears necessary before the flesh can be ingested by the phagocytic cells which line the gut. Digestion in the Polycladida has never been studied; conditions probably resemble those in the Tricladida. The Rhabdocoelida have been examined only by Westblad (1923), who finds the same conditions except in members of the Hysterophora (with the exception of *Prorhynchus*), where digestion is apparently largely, if not exclusively, extracellular. Unlike the other Turbellaria the epithelial cells of the gut are never amoeboid and never form syncytia, while the lumen of the gut is always large.

In *Limulus*, which feeds largely on bivalves and worms which are held under the mouth by the mouth-parts and shredded out by the gnathobases of the walking legs before being swallowed, digestion is primarily extracellular. Schlottke (1934*a*, 1935) has shown that the stomach juices contain a proteinase, a carboxypolypeptidase, an amylase and a lipase. Digestion of protein is completed *intracellularly* within the cells of the tubules of the digestive diverticula which contain a powerful dipeptidase. In view of their food and mode of feeding, closely analogous with that of decapod Crustacea where digestion is exclusively extracellular, this retention of intracellular digestion in *Limulus* may be regarded as primitive. Schlottke's general views on this matter are discussed below.

(2) In this group may be included the Brachiopoda, Rotifera, Tardigrada, Pycnogonida, Arachnida (other than *Limulus*) and the majority of the Mollusca excluding the Cephalopoda.

The Brachiopoda are all ciliary feeders. Little work has been done on them but sections of *Waldheimia* and *Lingula* (personal observations and those of Mr J. F. Sloane) fail to show any evidence of secretion in the gut, the digestive diverticula being apparently exclusively concerned with ingestion and intracellular digestion of the finely divided food. This apparent failure to develop the capacity for extracellular digestion may be one of the reasons why the Brachiopoda have been so unsuccessful. The Tardigrada apparently resemble them in the lack of extracellular digestion; as shown by Marcus (1935) they feed by sucking in soft plant tissues and ingesting the chloroplasts in the large cells of the mid-gut. In the Rotifera, which also feed by ciliary mechanisms, Remane (1929) and Beauchamp

(1932) have shown that some extracellular digestion precedes intracellular digestion.

In the Pycnogonida, which feed by means of a sucking proboscis sucking in soft tissues and fine particles (see review by Calman, 1929), Schlottke (1933*b*) has found that the epithelium of the mid-gut possesses secretory and absorptive cells (also embryonic cells), the former being responsible for the secretion of extracellular enzymes, the latter for ingestion and intracellular digestion, notably of proteins.

Amongst the Arachnida, digestion in the scorpions has been most recently studied by Pavlovsky & Zarin (1926) and Schlottke (1933*b*), who refer to earlier literature. In these animals also the contents of the body of animal prey are sucked up, after some preliminary extra-intestinal digestion of protein by extruded protease which converts them into fluid and minute particles. Pavlovsky & Zarin investigated the structure of the gut and also the nature of enzymes obtained from extracts, and Schlottke has definitely established the intracellular digestion of protein. In the false-scorpions (Chelonethi) Kaestner (1927) has established the presence of intracellular digestion, and Schlottke (1933*c*) the extra-intestinal liquefaction of the flesh of the prey. In the true spiders (Araneae), which also suck up the fluid juices of their prey, the presence of intracellular digestion was revealed originally by Bertkau (1884) and confirmed by Millot (1926) and Schlottke (1934*a*). Similar conditions have been observed in the Acari, *e.g.* in *Ornithodoros* by Christophers (1906), in *Liponyssus* by Reichenow (1918), and *Ixodes* by Roesler (1934). Apparently some degree of intracellular digestion occurs in all Arachnida.

In the Lamellibranchia (Yonge, 1923, 1926*a*, 1926*b*; for other literature see these papers and Yonge, 1928*a*) digestion of protein and fat is exclusively intracellular, the only extracellular digestive enzymes being those set free when the head of the crystalline style is dissolved in the stomach. These act on carbohydrates, principally starch. The tubules of the digestive diverticula (Yonge, 1926*a*) consist of cells concerned with the absorption of glucose and the ingestion and intracellular digestion of minute particles, with the addition of young cells which replace these as they are lost. With but few exceptions finely divided food is collected and sorted by means of ciliary mechanisms on the gills, palps and in the stomach. In *Teredo* and *Bankia*, particles of wood scraped off by the modified shell valves are ingested and some of the cellulose digested in a modified region of the digestive diverticula (Sigerfoos, 1908; Potts, 1923; Yonge, 1926*a*). The Septibranchia are carnivorous and the stomach has been converted into a crushing gizzard, but they continue to digest protein intracellularly in the tubules of the digestive diverticula which are unusually wide (Yonge, 1928*b*). In the Tridacnidae, though ciliary feeding mechanisms remain, much of the food consists of the zooxanthellae which occur in vast numbers in the thickened mantle lobes (Yonge, 1936*a*). A notable feature about digestion in the lamellibranchs, with the exception of the Protobranchia and the Septibranchia, is the part played by wandering phagocytic cells, the significance of which will be discussed in the next section.

Conditions in the Gastropoda are of particular interest. In these, unlike the

Lamellibranchia, occur feeding mechanisms of almost every type for utilizing all manner of food, with corresponding morphological and physiological adaptations in the gut. Moreover, there is a general tendency throughout the class for intracellular to be replaced by extracellular digestion. The herbivorous prosobranchs may be taken as primitive (the Polyplacophora being exclusively herbivorous). Conditions in these resemble in many ways those in the Lamellibranchia. The buccal ("salivary") glands secrete mucus, probably in connection with the working of the radula, as in *Patella* (Graham, 1932). The only extracellular enzyme is an amylase produced by the lateral diverticula of the foregut in *Patella*, and set free from the style in genera such as *Crepidula* (Mackintosh, 1925; Yonge, 1925*b*) and *Pterocera* (Yonge, 1932). Absorption and all digestion of proteins and fats takes place in the cells of the digestive diverticula. There are no wandering phagocytes. In the carnivorous prosobranchs (Hirsch, 1914) extracellular proteases occur, set free either by secreting cells in the digestive diverticula, *e.g.* *Pterotrachea*, or by the buccal glands as in *Natica*, or by both, *e.g.* *Murex*. Sucroclastic enzymes are secreted by the digestive diverticula, except in *Murex*, where they originate in the buccal glands. Intracellular digestion, and also absorption, takes place in the absorptive cells of the digestive diverticula in *Murex*, as shown by Hirsch (1924). Mansour-Bek (1934), working on the same genus, was, however, unable to distinguish any significant variations between the content of different grades of proteoclastic enzymes in extracts of the various buccal glands, digestive diverticula and in the stomach juices.

In the Opisthobranchia very similar conditions prevail. Hörstadius (1933) has demonstrated conclusively that intracellular digestion occurs both in the carnivorous tectibranch, *Pleurobranchia*, and in the herbivorous nudibranch, *Hermeae*, confirming in the latter instance the earlier observations of von Bruel (1904). Similar observations have been made in the case of *Doris* and various gymnosomatous Pteropoda (Yonge, 1931*b*). In the Pulmonata, however, conditions are different. Jordan (1918) originally claimed that phagocytosis occurred in the digestive diverticula of *Helix*, and Peczenik (1925) came to similar conclusions with *Limnea*. This was denied for *Helix* by Krijgsman (1925, 1928) and more recently, and very convincingly, by Hörstadius (1933). Jordan, discussing Hörstadius's then unpublished results and other relevant literature (see his review, 1932), concludes that in *Helix* (which may be considered typical of all pulmonates) digestion of proteins is probably intermediate between the intracellular and extracellular condition, because not even the finest particles enter the cells (as shown by Hörstadius); but complete digestion of proteins has not been demonstrated in the lumen of the gut (Graetz, 1929), and this probably takes place intracellularly after the soluble peptides have been absorbed.¹

The general problem of intracellular digestion in the Metazoa has been well reviewed by Hirsch (1925) and Jordan & Hirsch (1927). Schlottke (1934*b*) has put forward the view that intracellular digestion is confined to those animals in which

¹ Shinoda (1930) states that somewhat similar conditions prevail in the silkworm, *Bombyx mori*. He is the only worker who has found evidence of intracellular digestion in the Insecta.

the phagocytic regions of the gut develop from embryonic cells containing yolk, whereas in those groups of animals which digest extracellularly the portions of the gut concerned with absorption "alle resorbierenden Teile sich aus besonderen Anlagen bilden, die aus dotterlosen oder zum mindestens dotterarmen Zellen bestehen". He adds that the capacity for phagocytosis, including the taking in of soluble undigested protein, cannot be regained once it has been lost. He bases his views to a large extent on his statement that the kind of food and mode of feeding bears no relation to the mode of digestion, one type of digestion or the other alone being found in any group.

It is difficult to accept this contention. As indicated above, the presence of intracellular digestion in the coelenterates, Turbellaria and the most primitive living arachnid, *Limulus*, may be regarded as a retention of a primitive character. The great development of extracellular digestion in one section of the Rhabdocoe-lida is striking evidence of evolution within one of these groups. There is good evidence that intracellular digestion persists in other groups owing to their mode of feeding on finely divided food collected by ciliary mechanisms, *e.g.* the Brachiopoda, Rotifera and Lamellibranchia, or by the radula or gills in more primitive herbivorous Gastropoda, or else on fluid food which is sucked up, frequently after preliminary extra-intestinal digestion of protein, *e.g.* the Tardigrada, Pycnogonida and Arachnida. The presence of this sucking habit in all the arachnids other than the primitive *Limulus* appears a probable reason for the retention of some degree of intracellular digestion in this class, digestion in all other classes of the Arthropoda being apparently exclusively extracellular. Moreover, there is a notable difference between conditions in the simplest and the most complex Gastropoda, digestion being primarily intracellular in *Patella* and almost exclusively extracellular in *Helix*. A point of interest is that, both in Arachnida and Gastropoda, it is the digestion of proteins, requiring four sets of enzymes for its completion, which is the last completely to be emancipated from the intracellular condition.

III. DIGESTION IN AMOEBOCYTES

The various properties possessed by the amoebocytes of the Invertebrata have recently been well reviewed by Haughton (1934). That of particular significance here is the capacity for direct ingestion and digestion of food particles. This occurs in both the Lamellibranchia and the Echinodermata. In the former the presence of amoebocytes with phagocytic powers around the stomach, digestive diverticula and mid-gut, and also within the epithelia of these, was observed by a number of early workers, and it was later shown (Yonge, 1926*a*, 1926*b*) that these actually pass into the lumen of the gut and there ingest particles of food which they later carry back into the tissues and digest. Under certain circumstances these phagocytes may be extruded into the mantle cavity and there ingest food in the same manner (Yonge, 1928*c*; Koller, 1930), which provides the explanation for certain previous assertions that Lamellibranchia are capable of absorption through the epithelium of the mantle and gills. Takatsuki (1934) has studied the digestive power of these amoe-

bocytes and shown that in *Ostrea* they possess sucroclastic, lipoclastic and proteoclastic enzymes, and that they are also capable of absorbing glucose. They do not occur in the Septibranchia (Yonge, 1928*b*) where the food is not finely divided, and it is questionable whether they occur in the Protobranchia (which may be one reason for the small size of this class), but they are present in all Filibranchia and Eulamellibranchia which have been studied. In the Tridacnidae zooxanthellae are invariably contained within these cells, both when flourishing and dividing in the mantle edges and later when carried to the region around the digestive diverticula for digestion (Yonge, 1936*a*). There can be little doubt that the presence of this accessory means of intracellular digestion, which permits the ingestion of diatoms and other food particles too large to enter the tubules of the digestive diverticula, is of the greatest value to the Lamellibranchia and provides one reason why this highly organized and very successful class has developed extracellular digestion to such a very limited extent.

The conditions of digestion in the Echinodermata are by no means clear. The most important papers are those of van der Heyde (1922), van der Heyde & Oomen (1924), Oomen (1926), Weese (1926), and Sawano (1928). Extracellular digestion is certainly well developed, extracellular proteases being particularly powerful in the carnivorous Asteroidea and all types of enzymes in the omnivorous Echinoidea and Holothuroidea. In the Echinoidea and Holothuroidea, amoebocytes occur in the epithelium of the gut and also free in the lumen, and it is possible that these may be the principal agents of absorption and of some intracellular digestion. In the Asteroidea also there is intracellular digestion by means of phagocytes in the pyloric caeca, while Heyde & Oomen (1924) also report the ingestion of Indian ink particles by the cells of the intestinal caeca. It is difficult to be certain to what extent intracellular digestion, apart from that in phagocytic amoebocytes, occurs in the Echinodermata.

IV. DEVELOPMENT OF EXTRACELLULAR DIGESTION

Extracellular digestion, as already shown, occurs to a greater or lesser extent in all Metazoa with the exception of the Porifera and (probably) the Brachiopoda. Originally developed with the increased size of available food as an aid to intracellular digestion, particularly where no mechanism exists for the mechanical trituration of the food, it has eventually completely replaced intracellular digestion in certain rhabdocoel Turbellaria (probably), the Nematoda (Schuermans Stekhoven & Botman, 1930, 1932; Schuermans Stekhoven, 1931), the Polyzoa (Cori, 1929, 1930), the Annelida (Jordan, 1904*a*; Brasil, 1904; Nicol, 1930), the Myriapoda (Randow, 1924), the Crustacea and Insecta (numerous workers, see reviews of Jordan, 1913; Jordan & Hirsch, 1927; Yonge, 1931*b*; Kruger, 1934), the Cephalopoda (previous by no means extensive work is summarized in the reviews cited above; forthcoming work by Miss A. M. Bidder will remedy this deficiency),¹ the Tunicata (Yonge, 1925*a*; Berrill, 1929) and all higher Chordata.

¹ Unless otherwise stated, all references to the Cephalopoda are based on personal communications from Miss A. M. Bidder. The author wishes to acknowledge her valuable assistance.

The advantages of extracellular digestion are clear. Digestion outside the cell enables an animal to reduce the size of the alimentary system because intracellular digestion necessitates a large ingestive surface, *e.g.* the flagellated chambers in the Porifera, the mesenterial filaments of the Anthozoa, the ramifications of the gut in the Turbellaria and Trematoda, the hepatic caeca in the Asteroidea, the ramifications of the digestive diverticula in the Brachiopoda, Arachnida, Lamellibranchia and Gastropoda. Extracellular digestion also enables an animal to digest and, of equal importance, to get rid of indigestible matter, more rapidly. In this way metabolism is greatly assisted, and it is notable that the most active groups of animals, the Annelida, Crustacea, Insecta, Cephalopoda and Chordata, all digest exclusively in this manner. Moreover, with the exception of the Cephalopoda, these are the groups which have most successfully colonized the land surface. The development of extracellular digestion has had a profound effect on the evolution of the Metazoa.

The appearance of extracellular digestion has had far-reaching effects on the structure and physiology of the gut. Distinct regions have become specialized for secretion, absorption and for defaecation. Digestive enzymes have been evolved for special purposes. The time of secretion has been controlled in various ways. Various mechanisms ensure the correct hydrogen-ion concentration for the working of the enzymes in various regions of the gut.

(1) *Specialization of the gut*

The structure of the gut is modified greatly in correlation with the feeding habits and type of food. The terms fore-gut, mid-gut and hind-gut have primarily a purely morphological significance; considered from the *functional* aspect the gut can most conveniently be divided into the five regions given below. All associated structures are considered, although, of course, these are never all present in any one animal.

(a) *Region of reception.* This includes the mouth with associated feeding mechanisms (although these may be outside the mouth, *e.g.* the gills of the Lamellibranchia or the mouth-parts of Arthropoda), the buccal cavity (in which the feeding mechanism may lie, *e.g.* the radula), and the pharynx (or a "sucking stomach") which is notably developed in animals which feed by drawing in soil (*e.g.* Oligochaeta) or by sucking in fluid or soft tissues (for details see Yonge, 1928*a*, pp. 68-71). Glands are frequently present in this region of the gut opening into the buccal cavity or at the end of mouth-parts. These are frequently referred to as "salivary" glands, but, in view of the great variation in the nature of the secretion produced by them, topographical names are probably better, *i.e.* buccal glands if they open into buccal cavity, sublingual if under the radula (Gastropoda, Cephalopoda), labial glands where they open on the labium (many Insecta), pharyngeal glands (Turbellaria, Nematoda, Oligochaeta, Araneae), etc. The term "salivary gland" may more suitably be confined to the Vertebrata. The primitive function of these glands probably has to do with lubrication. They secrete mucus in the triclad Turbellaria, in Gastropoda such as *Patella*, in the Cephalopoda, in the earthworms (Keilin,

1920), and a fluid for moistening the food in many Insecta. But with the increasing development of extracellular digestion, they frequently acquire a digestive function, as in those Rhabdocoelida which digest extracellularly and in the more highly specialized Gastropoda and Insecta. The secretion produced may be concerned with the preliminary digestion of protein, *e.g.* Nematoda such as *Proleptus* (Schuurmans Stekhoven and Botman, 1930, 1932), many carnivorous Gastropoda (Hirsch, 1914), and possibly in the earthworms (Willem & Minne, 1899), or of carbohydrates as in *Helix* and other herbivorous Pulmonata and Opisthobranchia and in a variety of Insecta, *e.g.* amylase in the cockroach (Swingle, 1925), invertase in some Lepidoptera (Stober, 1927) and bees (Kratky, 1931). The secretion from these glands may be extruded into the body of the prey while this is held against the mouth, a condition known as extra-intestinal digestion. The extruded enzymes may, however, be secreted by glands of the mid-gut (*e.g.* Cephalopoda; some Coleoptera none of which possess labial glands), the actual secreting surface of the stomach may be extruded in certain Asteroidea, while the flesh-eating maggots discharge proteoclastic enzymes with the faeces (Hobson, 1931 *a, b*, 1932). The subject of extra-intestinal digestion has been reviewed by Jordan (1910) and von Lengerken (1924).

The secretion of these glands may have a variety of other functions. In certain carnivorous Gastropoda (*Dolium*, *Cassis*, etc.), which do not possess a crushing gizzard, the buccal glands produce acid for the dissolution of calaceous matter in the food (Semon, 1889). In a variety of blood-sucking animals, *e.g.* Hirudinea, the annelid *Ichthyotomus* (Eisig, 1906), certain ticks (von Künssberg, 1911), and dipterous Insecta (Cornwall & Patton, 1914), they produce an anticoagulin. A poisonous secretion may also be produced, as in the *Toxiglossa* amongst the Gastropoda. This was originally thought to be the sole function of the posterior pair of buccal glands in the Cephalopoda, but Sereni (1929) came to the conclusion that the substance allied to tyramine found in these glands is also passed into the blood stream of the animal and is in the nature of a hormone. Sawano (1935) has recently found a dipeptidase and an amino-polypeptidase in extracts of these glands, but as he also found these in extracts of the crop and caecum, which do not secrete, it seems probable that they are autolytic enzymes; in any case it would be strange, as Sawano admits, if only the enzymes concerned with final, and not with preliminary, digestion of protein were secreted into the buccal cavity. Extra-intestinal digestion, which occurs in certain Octopoda, where it is probably correlated with the presence of a crop, is probably due to the regurgitation of enzymes from the gastric regions, the anterior buccal glands producing mucus only (though Sawano has found dipeptidase in extracts).

It should be noted that buccal glands do not occur in animals which collect finely divided food, *e.g.* the Lamellibranchia, or where there is internal trituration accompanied by a mixing with enzymes which originate in more posterior regions of the gut, *e.g.* the Crustacea.

(b) *Region of conduction and storage.* This comprises the oesophagus (regarded functionally as the region of the gut which leads to the site of final digestion; thus in both Lamellibranchia and Crustacea the mouth opens directly into the oesophagus),

which may or may not be dilated to form a crop. The latter is an organ in which food is stored previous to digestion, as in the herbivorous birds, and in many blood-sucking animals such as the Hirudinea where it comprises the major part of the gut, and many Insecta (see Wigglesworth, 1934, for a general account of the varied conditions here). Preliminary digestion may occur within it as in earthworms (if Willem & Minne, 1899, are correct in their statement that the pharyngeal glands produce proteoclastic enzymes), in many Insecta such as the cockroach where there is regurgitation of enzymes from the stomach (Sanford, 1918; Eidmann, 1924), and in herbivorous Gastropoda such as *Aplysia* and *Helix*. With the exception of animals which suck blood or other fluids, the crop is usually followed by a gizzard (e.g. birds, earthworms, insects such as the cockroach, *Aplysia*), but as this organ is more widespread and is associated normally not only with trituration but also with the mixing of the food with digestive enzymes, it will most conveniently be discussed below.

(c) *Region of digestion and internal trituration.* Under this heading are included the true stomach and other regions concerned with the final stages of digestion, and also regions, morphologically anterior to these but often in intimate functional association with them, which are concerned, as stated above, with trituration in cases where this does not take place in more anterior regions or is only incompletely performed there. In addition to the animals cited above, a gizzard is present in the Rotifera and also in the Septibranchia, where the stomach has become very muscular and is covered with a thick cuticle which probably represents the extension of the gastric shield present in other Lamellibranchia (Yonge, 1928*b*). The very muscular pharynx of the annelid *Aphrodite* is essentially a crushing gizzard (Jordan, 1904*a*). In the Crustacea the so-called stomach, which is ectodermal and lined with chitin, consists of a large cavity in which food is mixed with enzymes produced in the morphological mid-gut. This region is not very complex in the setous-feeding Entomostraca, but in the higher groups, notably the Decapoda, extensive trituration takes place as a result of the action of the calcareous teeth in the gastric mill. The posterior regions of the gizzard often possess filters which allow only finely divided material or fluid to pass through. This is the case in many Insecta, in *Aphrodite*, and in many Crustacea. In the last-named the filters are very complex, notably those concerned with guarding the entrance to the digestive diverticula ("hepatopancreas") in which absorption (as well as secretion, see below) takes place. The nature and function of these filters has been described in *Nebalia*, *Idothea*, *Gammarus* (Jordan, 1909), *Asellus* (Rehorst, 1914), *Ligia* (Nicholls, 1931), *Astacus* (Jordan, 1904*b*), *Homarus* (Williams, 1907) and *Nephrops* (Yonge, 1924). In the Lamellibranchia the stomach is the site of extracellular digestion by the enzyme liberated from the style and, in the Filibranchia and Eulamellibranchia, of intracellular digestion by the phagocytic blood cells, but it is also concerned with the final sorting of the finely divided food, only the finest particles being passed into the ducts of the digestive diverticula, the larger ones being conducted directly into the intestine. Special sorting regions have been described in the stomachs of *Modiolus* (Nelson, 1918), *Mya* (Yonge, 1923), *Ostrea* (Yonge, 1926*b*) and *Ensis* (Graham,

1931a). Similar sorting mechanisms exist in the stomach of many herbivorous Gastropoda, e.g. *Pterocera* and *Vermetus* (Yonge, 1932). In the Cephalopoda where the stomach is concerned with digestion there is a collecting mechanism in the caecum, the function of which appears to be the removal of solid particles, leaving fluid to be absorbed (see below).

Digestive enzymes are secreted either by unicellular glands lining the digestive regions or by diverticula from these in which absorption may or may not also take place (see below). In the Coelenterata secreting cells are scattered about in the endoderm (Hydrozoa; Beutler, 1924, 1926), or are localized in the gastric filaments (Scyphozoa; H. G. Smith, 1936) or in the mesenterial filaments (Anthozoa; Boschma, 1925; Yonge, 1931a). In the Echinodermata, secretion takes place exclusively in the stomach of the Asteroidea (van der Heyde, 1922; van der Heyde & Oomen, 1924) and in the stomach regions of the gut in the Echinoidea (van der Heyde, 1922) and the Holothuroidea (Oomen, 1926). The same conditions prevail in the Polyzoa (Cori, 1929, 1930) and in the majority of the Annelida (Brasil, 1904; Nicol, 1930), although in *Aphrodite* secretion is confined to the lateral diverticula from the gut (Jordan, 1904a), while in earthworms of the genus *Eutyphoeus*, Bahl & Lal (1933) have found five pairs of glands in the intestine. The Nematoda can be divided into those like *Ascaris*, where the intestine both secretes and absorbs, and those like *Proleptus*, where secretion takes place in pharyngeal glands and the intestine is concerned exclusively with absorption (Schuermans Stekhoven & Botman, 1930, 1932; Schuurmans Stekhoven, 1931). In the Tunicata secretion may take place in the stomach as in *Ciona* (Yonge, 1925a), or there may be diverticula ("liver") which are the primary organs of secretion, as in *Tethyum* and *Boltenia* (Berrill, 1929). Secretion occurs in diverticula of one kind or another in the Rotifera, the Mollusca (confined in the Lamellibranchia to the style-sac) and in the Arthropoda, although in the Insecta the enteric caeca are no more than extensions of the stomach surface with similar secreting and absorbing functions.

The function of these diverticula varies greatly in different phyla, and even in members of the same phyla, notably the Mollusca. They may be concerned with intracellular digestion alone, e.g. the polyclad Turbellaria, the Brachiopoda (probably) and the Lamellibranchia and certain Gastropoda; with absorption (and intracellular digestion by means of amoebocytes) as in the pyloric caeca of the Asteroidea; with secretion and intracellular digestion as in the Arachnida and most Gastropoda; with secretion and absorption as in the Arthropoda and *Aphrodite* (and possibly *Nautilus*); and finally with secretion alone as in the Cephalopoda (both "liver" and "pancreas") and in such Tunicata as they occur (and also, of course, in all Vertebrata). They have received many names, e.g. liver (the result of the original discovery by Claude Bernard that the diverticula in Mollusca resemble the liver of vertebrates to the extent that they contain glycogen), hepatopancreas, gastric gland, etc. Jordan (1912) has discussed the "Leberfrage" in the invertebrates and shown the erroneous character of the names given to the diverticula. Yonge (1926a) suggested that the name digestive diverticula be given to these organs in the Lamellibranchia, a name giving a general indication of their function and structure.

This name has been widely used by subsequent workers on this class. It is here used for organs of this character in Lamellibranchia, Gastropoda, Arachnida and Crustacea, and its general application is advocated in view of the very varied functions which the diverticula possess.

The digestive region, no matter whether this be localized in diverticula, *e.g.* Mollusca and Crustacea, distributed between the stomach and diverticula, *e.g.* Insecta and Arachnida, or confined to a restricted portion of the straight gut, *e.g.* many Annelida, and both when it is concerned with intracellular and with extracellular digestion, has certain general properties. It is characterized by the presence of embryonic cells which, as a result of frequent divisions, are able to replace the older, functional cells, which eventually degenerate and are discharged into the lumen of the gut. Thus Gutheil (1912) and Yonge (1926*a*) have observed the presence of mitotic division in the "crypts" of young cells in the diverticula of Lamellibranchia, Brasil (1904) nuclear division in the secreting region of the gut in a variety of Polychaeta, Schlottke (1933*b*) embryonic cells in the mid-gut of Pycnogonida, Jacobs (1928) cell division and the formation of young cells at the distal ends of the diverticula in *Astacus*, and Faasch (1935) crypts of young cells in the mid-gut of the flea, *Hystrichopsylla*.

The fate of these young cells varies. In the Lamellibranchia they develop into one type of cell only which is concerned with absorption and with intracellular digestion and which finally, when full of the indigestible residue after intracellular digestion, is passed into the lumen (Yonge, 1926*a*). In the decapod Crustacea, as exemplified by *Astacus*, the young cells in the diverticula may become either absorptive or secretory. Hirsch & Jacobs (1930) have shown that if meat coloured with lithium carmine is fed to *Astacus* the carmine later appears in many of the cells in the diverticula. In the case of the true absorptive cells it is later passed into wandering cells, but if the cells which absorb it are in the process of conversion into secretory cells the carmine is retained within them and later extruded with the secretion. The act of secretion ends the life of these cells; secretion is holocrine, the cells being, in the terminology of Hirsch (1931), monophasic. In the Insecta secretion may be holocrine, *e.g.* Orthoptera, or merocrine, *e.g.* Lepidoptera (Shinoda, 1927). In the latter case, where the same cell can continue to secrete rhythmically for long periods (*i.e.* is polyphasic according to Hirsch), periods of activity alternate with periods of quiescence. In the larvae of Lepidoptera, Shinoda (1926) states that *all* the cells of the digestive region are similar, passing successively through the following stages: secretion, senescence, rejuvenescence (goblet cells which neither secrete nor absorb), absorption, the cycle of changes then starting over again.

(*d*) *Region of absorption.* This region also, as already noted, frequently overlaps the previous one. Jordan (1927) has reviewed work on absorption in the invertebrates. In animals which digest intracellularly both digestion and absorption must occur in the same cells, but with the development of extracellular digestion the two processes become separated. They may, however, be carried out by different stages in the same cells, as just recorded for the larvae of Lepidoptera, and also in other Insecta (Steudel, 1913). Digestive and absorptive cells may be mingled together

in the same region, *e.g.* in Nematoda resembling *Ascaris*, in the intestinal caeca in *Aphrodite* amongst the Polychaeta, possibly throughout the intestine in the Oligochaeta (see Stephenson, 1930, for references), in the stomach and diverticula or enteric caeca in the Arachnida and Insecta, and in the digestive diverticula in the pulmonate Gastropoda (in the lower Gastropoda, as in the Lamellibranchia, absorption is carried out by the ingestive cells in the diverticula). In the simpler Crustacea, such as *Calanus* (Lowe, 1935), secreting and absorptive cells occur together in the mid-gut which is almost devoid of diverticula. With the development of diverticula, *e.g.* in the Isopoda (Murlin, 1902; Nicholls, 1931), the wide intestine remains at first the major site of absorption, though this also occurs to some extent in the diverticula. In the Decapoda there is a great increase in the diverticula and a reduction in the mid-gut which in the majority (*Nephrops*, *Paguristes* and *Homarus* are amongst the few exceptions) is extremely short (see Frenzel, 1884), the ectodermal hind-gut, which is lined with chitin and has no absorptive functions, being correspondingly long. Absorption is here practically confined to the diverticula.

In cases where food has to pass into the diverticula, and waste material to pass out, with or without secretions, mechanisms must exist for this two-way flow. In the Lamellibranchia the ducts leading into the diverticula are ciliated, allowing an inward passage on the one side and an outward passage on the other. The cells of the diverticula never appear ciliated when examined in sections, but by a study of fresh material Potts (1923) has shown the presence of long retractile cilia in three genera, which has been confirmed by Yonge (1926*a*) for five others. These cilia maintain a circulation within the diverticula. In the Crustacea the diverticula are surrounded by a network of striated muscle fibres, a very detailed account of which has been given by Pump (1914). Contraction of the fibres arranged circularly causes expulsion of the digestive enzymes which are passed by definite channels into the "stomach" (Jordan, 1904*a*; Yonge, 1924), while relaxation of these and contraction of the longitudinal fibres causes the drawing in of digested material which is strained through the filters in the "pyloric" end of the "stomach".

In the Echinodermata, absorption appears to be confined to the pyloric caeca in the Asteroidea and to take place in the intestinal region of the gut in the Echinoidea and Holothuroidea. The properties of the gut of the last-named have given rise to considerable controversy; Cohnheim (1901) regarded it as a simple diffusion membrane but capable of absorbing; Enriques (1903) stated that it is semi-permeable and cannot absorb; Oomen (1925) agreed with Enriques, explaining Cohnheim's results by stating that the gut became a simple diffusion membrane after death; finally Schreiber (1930) decided that the living gut can absorb, but that after removal from the body (under which conditions previous observations had been made) it becomes first semi-permeable and then freely permeable.

In Nematoda resembling *Proleptus* the intestine is concerned exclusively with absorption. In many polychaetes, such as *Sabella* (Nicol, 1930), the gut is straight but can be divided into four regions histologically, oesophagus (conducting), stomach (secretion of digestive enzymes), intestine (absorptive) and rectum (con-

ducting, with formation of faeces). In the Hirudinea, absorption is probably confined to the short intestine. Absorption in the dibranchiate Cephalopoda occurs in the caecum and intestine. In the Tunicata, as exemplified by *Ciona* (Yonge, 1925 *a*), absorption takes place primarily in the intestine, and to a slight extent in the stomach and in the rectum. In the vertebrates it is, of course, confined to the intestine.

A few anomalous cases of absorption are worthy of note. Although the gut of *Helix* does not absorb (Jordan & Lam, 1918), it is a diffusion membrane which allows even disaccharides to pass through it (Jordan & Begemann, 1921). Fats are absorbed through the walls of the crop in the cockroach (Sanford, 1918; Abbott, 1926), the lipase being secreted in the stomach region and regurgitated through the gizzard. No other substance can be absorbed through the chitinous intima, and passage of fatty acids is presumably due to presence of lipid material in the epicuticula of the lining integument, Yonge (1936 *b*) having shown that the uncalcified integument of Crustacea, the bounding cuticle of which also contains a lipin, is permeable to fatty acids and other fat solvents.

In regions where chitin *alone* bounds the gut, *e.g.* the peritrophic membrane of Insecta (Wigglesworth, 1929) and the intestine of Isopoda—the origin of which embryologically is obscure (Murlin, 1902; Nicholls, 1931)—absorption takes place through this. In some cases absorption may also take place in the hind-gut in Insecta, *e.g.* in lamellicorn larvae (Wiedemann, 1931), where micro-organisms which feed on the cellulose in the food are here digested and the products of digestion absorbed in special areas of cells.

(*e*) *Region of conduction and formation of faeces.* With the exception of certain Insecta, as noted above, the hind-gut or rectum is concerned with the moulding of the faeces and their passage to the exterior by way of the anus. This usually involves the absorption of water, a matter of the greatest importance in Insecta where water must be conserved, and it is there frequently carried out by rectal glands (Wigglesworth, 1932). The faeces may be moulded in a comparatively short and very muscular rectum, as in the Arthropoda generally. In other cases the morphological mid-gut may also be concerned with faeces formation. As stated elsewhere (Yonge, 1935) there appears to be a correlation between the length and function of the mid-gut and the position where faeces are extruded. Graham (1932) has pointed out that the long coiled gut of *Patella* is probably concerned exclusively with the formation of firm faeces, and this will be equally true of the Lamellibranchia. In neither Gastropoda nor Lamellibranchia does absorption occur in the intestine, and it is essential where faeces are discharged into the mantle cavity that they should not be in such a condition that they will foul it. In both groups propulsion through the gut is by cilia, and for that reason the preliminary moulding of the faeces takes a considerable time. It is notable that in the carnivorous Septibranchia, the intestine is a straight tube leading directly from the stomach to the rectum. In the ascidians and in the ciliary-feeding annelids, in neither of which is there any danger of the faeces fouling the food-collecting mechanism or the respiratory system, the intestine is concerned with absorption and the rectum

alone with the faeces formation. In all cases the rectum possesses numerous mucous glands and is increasingly muscular as the anus is approached.

The firmness and characteristic, even specific, shape of the faecal pellets in a variety of invertebrates has been well shown by Moore (1931*a*, 1931*b*, 1932*a*, 1932*b*). In the Crustacea this is clearly due to the muscular action of the chitinized hind-gut, in the ciliary-feeding animals mentioned above in some cases to a preliminary moulding in the intestine but always to a final secretion of mucus in the rectum and the action of the muscle in this region. Attention has already been called (Yonge, 1935) to the high pH in this region of the gut which will have the effect of increasing the viscosity of the mucus and so adding to the firmness of the faeces.

(2) Digestive enzymes

Of recent years the digestive enzymes of many invertebrates have been studied with modern technique in considerable detail. This work has been reviewed by Krüger (1929, 1933, 1934) and Jordan (1932). The most important work has been done on the following groups. Hirudinea: Autrum & Graetz (1934) on the lipoclastic enzymes of *Hirudo* and *Haemopsis*, Graetz & Autrum (1935) on proteoclastic enzymes. Arachnida: Schlottke (1935) on the digestive enzymes of *Limulus*. Decapod Crustacea: Lowartz (1919) and Wiersma & Veen (1928) on the sucroclastic enzymes of *Astacus*, Shinoda (1928) on the proteoclastic enzymes, Vonk (1935) on the lipoclastic enzymes in *Astacus* and other Crustacea, Krüger & Graetz (1928) on all enzymes of *Astacus*, Mansour-Bek (1932) on the proteoclastic enzymes of *Maja*. Insecta: work on this group is reviewed by Uvarov (1928) and Wigglesworth (1934); the best general work of insect digestion is contained in the papers of Wigglesworth (1927*a*, 1927*b*, 1928, 1929) dealing with the cockroach and with *Glossina*, and that of Shinoda (1930, 1931) on the silkworm. Gastropoda: Graetz (1929) on the digestive enzymes of *Helix*, Kuntara (1934) on the lipoclastic enzymes and Rosén (1934) on the proteoclastic enzymes, Rosén (1932) on enzymes in a variety of Gastropoda, Mansour-Bek (1934) on the proteoclastic enzymes of *Murex*. Cephalopoda: Sawano (1935) on the proteoclastic enzymes of *Polypus*.

Of particular interest in this recent work is the division, by adsorption methods, of the proteoclastic enzymes of invertebrates into the "Teilenzyme", proteinase, amino-polypeptidase, carboxy-polypeptidase and dipeptidase, identified in the Mammalia by Waldschmidt-Leitz (1931) and his collaborators. These have been found in the Hirudinea (Graetz & Autrum, 1935), *Limulus* (Schlottke, 1935), *Maja* (Mansour-Bek, 1932), *Murex* (Mansour-Bek, 1934), *Helix* (Rosén, 1934) and *Polypus* (Sawano, 1935). In the Coelenterata extracellular digestion appears to be confined to the action of proteinase, dipeptidase being probably confined to intracellular action (Yonge, 1930*b*). The recent work of Vonk (1935) on fat digestion in *Astacus* is of interest because he shows that here, and in other decapod Crustacea, the digestive secretion is capable of emulsifying fats, a property which is confined in the vertebrates to the bile secreted by the liver.

There is very definite correlation between the food of any animal and the nature and relative strengths of its digestive enzymes. Thus omnivorous animals,

such as the Echinoidea, Holothuroidea, most Annelida, and the decapod Crustacea, digest carbohydrate, protein and fat with equal ease. Carnivorous animals, such as the Coelenterata, Turbellaria, Asteroidea, Stomatopoda, and Cephalopoda have very powerful proteoclastic enzymes, but sucroclastic enzymes are weak, the Madreporaria appear to be incapable of digesting starch (Yonge, 1930*b*) and Petrievic (1915) was unable to find either amylase or invertase in the Stomatopoda. Shinoda (1931) has shown that certain silkworms are unable to digest starch but that the blood contains desamidase which may enable the animal to synthesize carbohydrate from protein. In herbivorous animals proteoclastic enzymes are correspondingly weaker, *e.g.* in the Lamellibranchia and herbivorous Gastropoda (where a style is present these resemble Lamellibranchia in having no extracellular protease), the Cirripedia, where Patané (1928) was unable to find any protease (though some means of protein digestion must be present), and in the Tunicata. In the Insecta every type of feeding habit is found and the correlation between food and enzymes has been stressed by both Uvarov (1928) and Wigglesworth (1934), who provide tables comparing the food of different insects with the enzymes present in the gut.

Certain animals have acquired specific enzymes which have enabled them to exploit additional sources of energy. Of these the most important is cellulose. Amongst the Metazoa this enzyme is present in *Helix*, originally discovered by Biedermann & Moritz (1898), its properties have been extensively studied by Karrer and his school (*e.g.* Karrer & Illing, 1925). A very powerful cellulase is also present in the herbivorous prosobranch Gastropoda, *Pterocera* and *Strombus* (Yonge, 1932). As already stated, certain of the digestive diverticula of *Teredo* and the closely allied genus *Bankia* are specialized for the ingestion and intracellular digestion of wood particles scraped off by the modified shell valves during the process of boring. The presence of a cellulase has been established by Harington (1921), Dore & Miller (1923), Miller & Boynton (1926) and Boynton & Miller (1927). Cellulase is more widespread in the Insecta, having been found in certain locusts (Biedermann, 1919), in the stick insect, *Dixippus* (Bělehrádek, 1922), in the larva of the lepidopteran *Cemistoma* (Hering, 1926), in certain Capsidae and Coccidae (K. M. Smith, 1926), in the larvae of the beetle, *Hylotrupes bajulus* (Falck, 1930), in the larvae of a variety of other wood-eating beetles (Ripper, 1930) and in the larvae of another beetle, *Macrotoma plamata* (Mansour & Mansour-Bek, 1933, 1934*a*). The identification of cellulase in these insects has disproved to a large extent the contention of Buchner (1930) and his school that wood-eating insects depend on intestinal symbionts for the digestion of cellulase, while Mansour & Mansour-Bek (1934*a*) have shown that the larvae of *Xystrocera globosa* possess no cellulase but live in sapwood, which is comparatively rich in starch and provides them with the necessary carbohydrates. In other cases, *e.g.* lamellicorn larvae (Wiedemann, 1930) and possibly in the wood-eating termites (Mansour & Mansour-Bek, 1934*b*), the intestinal symbionts digest wood but are probably themselves digested by the insects. The whole subject has been dealt with in *Biological Reviews* by Mansour & Mansour-Bek (1934*b*).

Chitin is digested by a smaller number of animals. A chitinase occurs in *Helix* (Karrer & Hofmann, 1929; Karrer & von François, 1929). Among Insecta, Schulze (1927) has found chitinase in the beetle, *Platydemus tricuspidis*, which feeds on fungi, and Ramme (1920) in the larvae of the ectoparasitic hymenopteran, *Pseudagenia*, which feeds on spiders, the enzyme being used for the extra-intestinal digestion of the integument of the host. Ullmann (1932), in his work on the digestion by invertebrates of polysaccharides, was unable to find any digestion of inulin, but states that the digestion of lichenin is widespread. He also finds that invertebrates cannot utilize starch grains if these are not broken up mechanically by trituration, because they do not possess enzymes capable of digesting the investing layer of amylopectin. He considers that the chief sources of carbohydrates in plant food are soluble sugars and the so-called hemicelluloses.

In some Insecta unusual powers of protein digestion occur, notably in the larva of the blowfly, *Lucilia sericata*, which feeds on flesh and is capable of digesting collagen (Hobson, 1931*a*, 1931*b*, 1932), and in the larvae of the clothes moth, *Tineola biselliella*, where a reduction of the keratin in the hair (wool) on which the larvae feed opens its peptide chains to the attack of proteinase (Linderstrøm-Lang & Duspiva, 1935). The only unusual lipoclastic enzyme as yet identified is one present in the larvae of the moth, *Galleria melonella*, which feeds on the combs in beehives and is able to digest the wax (Sieber & Metalnikow, 1904; Fliessinger, 1910).

(3) Periodicity of secretion

The secretion of digestive enzymes into the lumen of the gut is controlled in a variety of ways. In the Coelenterata extracellular digestion is confined to the secretion, by glands on the mesenterial filaments, of a proteinase. Secretion appears to be stimulated by the close proximity of food material (of animal origin); it is difficult to obtain appreciable digestive action with fluid from the coelenteron except immediately after feeding with meat (Bodansky, 1924; Yonge, 1930*b*).

In the Gastropoda, Hirsch (1914) originally showed the presence of rhythmical secretion in the buccal glands and ferment cells of the digestive diverticula in *Murex*, *Natica*, *Pterotrachea* and *Pleurobranchia*. He has more recently (1931) summarized the results of this and later work on the same subject. The most detailed work is that of Krijgsman (1925, 1928), on both buccal glands and digestive diverticula in *Helix*. In the former, secretion is rhythmical, all cells working synchronously. It is merocrine, and after extrusion of secretion the cells go through a restitution process (Hirsch, 1931) involving the gradual elaboration of new secretion, the cytological changes involved being described in detail by Krijgsman. In a starved animal where secretion, though slow, never stops, but is asynchronous, he was able to identify some eleven stages. After feeding, the process becomes much quicker and synchronous owing to the shortening of the series of restitution changes, the process gradually becoming longer and synchronous action disappearing after the stimulus of feeding is removed. The ferment cells in the digestive diverticula also secrete rhythmically and always synchronously, so that whether stimulated or not the gland

always acts as a whole. Krijgsman was able always to distinguish four stages in the elaboration of secretion within the cells, although the speed of rhythm of secretion was quickened after feeding. In the case of the buccal glands the activating stimulus is bound up with the duration of feeding and may be concerned in the first place with smell, taste or the movement of the radula. Stimulus of the ferment cells of the digestive diverticula is *not* bound up with the duration of feeding but with the nature of the contents of the crop. In both cases the actual effect of the stimulation causing secretion is the increased taking up of fluid by the cells and increased permeability of the cell membrane. Ni (1933) has examined the excised buccal glands of *Aplysia* and found that they can be induced to secrete by electrical stimulation.

In the decapod Crustacea, Hirsch & Jacobs (1928, 1930) have shown that the digestive diverticula in *Astacus* secrete rhythmically, the individual cells working synchronously. Here, as already noted, secretion is holocrine and there is no restitution in individual secreting cells, although there is in the gland as a whole owing to the formation of new cells at the ends of the diverticula. As stated by Hirsch (1931), glands are always polyphasic although the actual cells may be polyphasic or monophasic. In *Astacus* rhythmical secretion is the ultimate result of rhythmical restitution owing to the formation of new cells by mitotic division. Rhythmical secretion occurs during starvation, but the process is speeded up greatly after feeding. There is also much greater activity in spring than in summer or autumn, the number and velocity of mitotic divisions being notably greater during the former period. It is probable that rhythmical secretion occurs also on the Insecta, Shinoda (1926) having noted a regular succession of stages in the cells of the mid-gut in the Lepidoptera, thereby confirming and extending earlier observations by Steudel (1913) on *Periplaneta* and *Carabus*.

Rhythmical secretion, which is greatly speeded up by the stimulus of food, is well suited to the needs of predacious carnivores, e.g. *Murex*, *Natica* or *Pterotrachea*, omnivorous scavengers such as the decapod Crustacea in general, or browsing herbivores such as *Helix* or *Aplysia*. But animals which feed on finely divided food which they collect continuously by means of ciliary or setous mechanisms (see Yonge, 1928*a*) need, to the extent to which they depend on extracellular enzymes for digestion, continuous secretion. Nothing is known of the periodicity of secretion in the setous-feeding Crustacea. In the ciliary-feeding Lamellibranchia and in style-bearing Gastropoda (see Yonge, 1932, for list of these), the only extracellular enzyme is that secreted in the style-sac where it is presumably adsorbed by the protein substance of the style and liberated in the lumen of the stomach when the head of the style is dissolved by the less acid nature of the fluid (Yonge, 1925*b*). As already emphasized (Yonge, 1932, 1935), the style constitutes an ideal mechanism for the continuous liberation of small quantities of enzyme (amylase). Even though there is a periodicity in the actual secretion of enzyme in the style-sac, the *liberation* of the enzyme into the stomach will be continuous. The substance of the style provides a perfect vehicle both for the conveyance and continuous liberation of the enzyme. All that is known about the actual secretion of the style (and enzyme)

is that this stops when the animals are taken out of water or in other ways experience unfavourable conditions such as lack of oxygen. This is indicated by the fact that under these conditions the style disappears in animals where the style-sac is in free communication with the gut. Normally there is a balance between the rate of secretion in the style-sac and the rate of dissolution by the less acid contents of the stomach; when secretion is lowered the latter process preponderates (Yonge, 1925*b*).

(4) *Factors controlling digestion*

The action of digestive enzymes is, above all else, dependent on the hydrogen-ion concentration of the medium. Wherever intracellular digestion has been studied, in the Protozoa or Metazoa, it has been shown to be carried out in two main stages, a preliminary acid phase when the food is killed and some preliminary digestion takes place and a later, alkaline, phase when digestion is completed.

Extracellular digestion involves the maintenance of the hydrogen-ion concentration in the lumen of the various digesting regions of the gut at such a figure as to make possible the action of the enzymes which work in those regions. A great deal of work, to which reference has already been made, has been done on the *pH* optima of the digestive enzymes of invertebrates, and observations on the *pH* in the gut have revealed that this is maintained at a figure at or very close to these optima. It remains to consider the various mechanisms whereby the *pH* of the gut is controlled.

In the Coelenterata the secretion of the extracellular proteinase is apparently accompanied by a substance which lowers the *pH* in the coelenteron. Thus the *pH* in the coelenteron of the madreporarian *Fungia*, is about 7·8 in a starving animal, but 2 hours after feeding with meat the *pH* falls to about 7·0, which is optimal for the working of the proteinase (Yonge, 1930*b*). Similarly in the scyphozoan, *Cassiopea*, Smith, H. G. (1936) has found that there is a similar drop in the *pH* after feeding and in association with the secretion of proteinase, and that some hours after feeding, when digestion is completed, the coelenteron is "flushed out" and the *pH* rises again.

Irving (1926) has studied the control of *pH* in the starfishes, *Patiria miniata* and *Asterias ochraceus*. The *pH* in the pyloric caeca is about 6·7, which represents optimal conditions both for the survival of cilia (concerned with transport in the gut) and for the extracellular digestion of protein. He found that this *pH* was maintained even after the caeca were excised, and came to the conclusion that a regulatory mechanism exists involving the control of the hydrogen-ion concentration by means of the carbon dioxide produced during metabolism.

In the earthworms the calciferous glands in the oesophagus which secrete crystals of calcium carbonate into the lumen may possibly be concerned with neutralizing the free acid in the earth and humus which is swallowed, and also assisting in the regulation of the hydrogen-ion concentration for digestive purposes (Dotterweich, 1933; Voigt, 1933; see Stephenson, 1930, for references to earlier work).

The *pH* of the stomach in Lamellibranchia and in Gastropoda with styles is

controlled by the style (Yonge, 1925 *b*, 1926 *b*). The entire gut has an acid reaction, the stomach being the most acid region, *e.g.* *Pecten*, pH 5.6; *Mya*, pH 5.8; *Ensis*, pH 5.7; *Ostrea*, pH 5.5. The style itself has in all cases a still lower pH, and it is the dissolution of this substance in the stomach which is responsible for the low pH. After the removal of the style from *Mya*, the pH rises to about 6.6, while subsection of *Ostrea* to unfavourable conditions which inhibits secretion of the style so that it eventually disappears (unlike *Mya* the style-sac in *Ostrea* lies in communication with the intestine) is accompanied by a rise in the pH of the stomach contents to about 6.15. A similar state of affairs prevails in the style-bearing gastropod, *Crepidula*, where the pH in the stomach rises from 6.0 to 7.0 as a result of the disappearance of the style (Yonge, 1925 *b*). In all cases the pH produced in the stomach as a result of the dissolution of the style is optimal for the action of the amylase in the style. Another point of interest is that in these animals there is a correlation between the pH of any region of the gut and the tolerance of the cilia in this region to the presence of hydrogen ions.

In other Gastropoda, such as *Helix*, the digestive diverticula may possess "calcareous cells" which, as Krijgsman (1928) has shown, secrete rhythmically both during feeding and starvation. They produce calcium phosphate which is probably concerned with the regulation of the pH in the crop.

In general the pH of the gut in herbivorous Insecta is more alkaline than in the carnivorous Insecta. Little is known, however, as to the manner in which this is regulated. Hobson (1931 *a*) considers that the acidity in the middle segment of the mid-gut of the larvae of *Lucilia* may be due to the secretion of phosphoric acid¹. In other cases the presence of micro-organisms influences the pH. Wigglesworth (1927 *a*) finds that the pH of the crop in the cockroach may fall as low as 4.8 after a meal on carbohydrates owing to acid formation by micro-organisms. In the larvae of certain lamellicorn beetles, such as *Potosia*, very interesting conditions have been found by Wiedemann (1930). These animals feed on rotting wood, taking in bacteria with the wood, while flagellates normally occur in the gut of the insects. The reaction in the stomach is alkaline, probably the result of secretions, in which medium the micro-organisms flourish. The reaction becomes increasingly acid in the enlarged proctodaeal chamber probably owing to the acid formed by the action of the bacteria. Proteoclastic enzymes are secreted in the stomach but are active only in acid media, so that they are unable to digest until they reach the posterior portion of the proctodaeal sac. Here they digest the micro-organisms, the bacteria having fed on the wood and the flagellates on the bacteria. The mode of absorption has already been mentioned (p. 100).

The varying pH values of the different regions of the gut may be of importance in ciliary-feeding animals by its influence on the viscosity of the mucus with which the food is entangled. There appears to be a correlation between the iso-electric point of the mucus in Lamellibranchia such as *Ostrea*, *Mytilus* and *Pecten*, in ascidians such as *Phallusia* and in the ciliary-feeding actinian, *Metridium*, and the

¹ The presence of free acid in the gut has been claimed by Gruvel (1893) for Cirripedia and by Nirenstein (1922) and Szarski (1936) for Oligochaeta.

pH in the stomach or coelenteron (Yonge, 1935). The lowered viscosity in these regions may assist in the digestive processes.

There remains for consideration the temperature factor. The importance of duration of enzyme action in connection with temperature has been emphasized by Berrill (1929), Graham (1931*b*) and Pantin (1932). In the animals studied, ascidians and lamellibranchs, they found that at all normal temperatures the time taken for food to pass through the gut corresponds to the duration of the enzyme experiments for which that particular temperature was optimal. While these workers have drawn attention to the important fact that the longer the duration of enzyme action the lower will be the apparent optimum temperature, it is unfortunate that the experiments should have been carried out on ciliary-feeding animals where the amount of enzyme present in the lumen at any time is so much less than the amount used in experiments (extracts of large quantities of digestive tissues or large numbers of styles). Moreover, in the lamellibranchs the time of passage through the gut bears little relation to the effective duration of enzyme action because the great part of the time is spent in the non-absorbing intestine and rectum.

V. SUCCESS ATTAINED BY ADAPTABILITY

The Metazoa may be divided into two groups. The first of these consists of animals possessing feeding and digestive mechanisms of such a nature that they can be adapted for the collection and digestion of many types of food. The major examples of animals of this type are the Annelida, the Crustacea, the Insecta and the Gastropoda (and also the Vertebrata). In the Annelida and the Gastropoda there are predacious carnivores adapted for the seizure of living prey and its rapid digestion, omnivores which swallow bottom deposits and earth or scrape the surface of rocks, etc., herbivores which browse on vegetation (these are not found in the Annelida), and ciliary feeders which collect finely divided material, of which phytoplankton is the most important constituent. Both Crustacea and Insecta have adapted their mouth-parts and other appendages for the capture of almost every type of food, correlated with which are appropriate modifications in the structure of the gut and the nature of the digestive processes. All of these groups contain many parasitic members which feed on the tissues or digested food of their hosts and in which the feeding and digestive mechanisms are correspondingly modified.

Animals of this type have achieved success by the aid of both morphological and physiological adaptations. The many forms which feeding mechanisms may take (see Yonge, 1928*a*) are due to morphological modifications. The best examples are provided by the Insecta in which the mouth-parts are capable of almost infinite variation, and the opisthobranch Gastropoda, which include members such as *Doris*, specialized for feeding on sponges, *Aeolidia* on actinians, *Sphaerostoma* (*Tritonia*) on alcyonarians, *Calma glaucoides* on the eggs of gobies, *Scaphander* on lamellibranchs, *Aplysia* on green weeds, *Pleurobranchus* on ascidians, while the gymnosomatous Pteropoda are carnivorous and the thecasomatous Pteropoda are typical ciliary feeders. A similarly diversified list could be made from the Crustacea in the light of the abundant recent work on feeding mechanisms in this class.

With these modifications in the feeding mechanisms are associated morphological adaptation affecting the structure of the gut, influencing the relative sizes of the various regions, the presence or absence of particular regions, *e.g.* a sucking pharynx, a crop, a crushing gizzard with or without filtering arrangements, etc. Physiological adaptations affecting the digestive enzymes have been of equal importance. The relative strengths of these varies, as already noted, in correlation with the nature of the food, *e.g.* in different Insecta where food varies so greatly, and amongst the Crustacea, *e.g.* in the omnivorous Isopoda and Decapoda, the herbivorous Cirripedia, and the carnivorous Stomatopoda. Additional enzymes have been acquired. The most notable case is that of *Helix*. This is the largest known genus of animals, and its outstanding success may well be due to its remarkable digestive powers. It is equally capable of digesting the cell walls of green plants by means of cellulase and the cell walls of fungi by means of chitinase, and is therefore able to utilize its food, whatever its nature, to the full. In the same way certain wood-boring Insecta, owing to a similar acquisition of a cellulase, have been able to penetrate into the heart-wood of trees where starch and soluble sugars are not present.

It is these groups of animals in which the feeding and digestive mechanisms are capable of great modification which—other things being equal—have most successfully invaded new habitats. For passage to the land it was essential that feeding mechanisms should be such that they could be used with equal efficiency in the new habitat. The Gastropoda and Crustacea which have taken to life on land have all marine representatives with similar feeding and digestive mechanisms, only the Annelida have become notably more specialized as a result of life on land. The possession of jaws capable of great adaptation was one of the factors which enabled the vertebrates to colonize the land so successfully.

VI. SUCCESS ATTAINED BY SPECIALIZATION

The second group of successful animals are those in which feeding and digestive mechanisms have been evolved which have enabled them to utilize one type of food with exceptional efficiency. The best examples are furnished by the Coelenterata, Turbellaria, Arachnida and Cephalopoda, which are all specialized carnivores, and by the Brachiopoda, Lamellibranchia and Tunicata, all of which possess highly complex ciliary-feeding mechanisms for the collection, primarily, of phytoplankton. Unlike members of the preceding group, very few of these animals have taken to a parasitic life, but several groups of parasites, notably the Trematoda and Cestoda, should be included here.

In spite of their great variation in external form, the Coelenterata are, in respect of their feeding and digestive mechanisms, a remarkably homogeneous group. They are all carnivorous, the prey being caught by the aid of nematocysts. Tentacles are chiefly employed for carrying food to the mouth, but in a few Anthozoa there is an interesting physiological adaptation. In the actinian, *Metridium* (Parker, 1905, 1928), and in various Madreporaria, *e.g.* *Fungia* and allied genera, and *Tridacophyllia* and *Merulina* (Yonge, 1930a), ciliary currents are reversed on the stimulus

of food (meat or glycogen) and so are able to carry this to the mouth as well as to perform their normal cleansing functions. It is interesting that the cilia react to the same stimuli as the tentacles, which never carry material not of animal origin to the mouth. Throughout the Coelenterata the digestive processes are the same, the action of a powerful extracellular proteinase being followed by intracellular digestion, sucrolastic enzymes, other than glycogenase, being notably weak or absent. The Turbellaria, apart from a few of the Rhabdocoelida, are also very standardized in their feeding and digestive processes, the Arachnida (including also the Pycnogonida), with the exception of the archaic *Limulus*, all suck in the contents of the bodies of their animal prey and all have apparently similar digestive processes; finally the Cephalopoda are equally specialized predacious carnivores.

The Brachiopoda and Lamellibranchia provide a striking instance of convergence between two different groups as a result of the elaboration of similar feeding habits, the Lamellibranchia being much the more successful. Orton (1914) has already pointed out the superiority of the feeding mechanisms of the Lamellibranchia, and to this may be added the much greater specialization of the gut and of the digestive processes in these animals. Particular attention should be drawn to the sorting mechanism in the stomach, the presence of a style which assists, by its rotation, in the passage of food through the gut, and by its continuous slow dissolution in the stomach provides a continuous supply of extracellular amylase and also ensures the optimal pH for the working of this. Finally the free passage of phagocytic amoebocytes into the lumen of the gut permits the digestion of food particles too large to enter digestive diverticula.

Animals so specialized in their feeding and digestive systems would appear to be incapable of further evolution in this respect. Actually the Lamellibranchs, otherwise a very homogeneous group, have made three attempts at further evolution of their feeding and digestive mechanisms, two being successful and the third, in the main, unsuccessful, but all of particular interest in this connection.

The first successful attempt has been responsible for the evolution of the Teredinidae, culminating in *Teredo* and *Bankia*. These animals make burrows in wood and, unlike the less specialized genus *Xylophaga*, are able actually to digest the wood. The greatly modified shell valves are not only the boring organs but also, so far as the consumption of cellulose is concerned, the feeding organs; the presence of a specialized region of the digestive diverticula provides another morphological adaptation, while the elaboration within this of an intracellular cellulase constitutes a physiological adaptation. The Teredinidae are a widespread and highly successful family.

The Tridacnidae constitute the second successful line of evolution. Here the possibilities of the phagocytic amoebocytes have been further exploited by the retention within them of zooxanthellae, which are farmed in the greatly enlarged mantle edges and eventually carried to the regions around the digestive diverticula (here greatly reduced in number) for digestion within these phagocytes. To permit of the zooxanthellae being fully exposed to the light, the mantle and shell have twisted in relation to the visceral mass and associated organs so that the free edges

of the mantle become dorsal instead of ventral, the animals resting on the ventral side (Yonge, 1936*a*). The organs of feeding have been modified to some extent. Members of this family are extremely abundant in the tropical Indo-Pacific.

The Septibranchia form the relatively unsuccessful group. Here an attempt has been made to adapt feeding and digestive mechanisms very highly specialized for the capture and digestion of finely divided food, largely of a vegetable nature, to the capture and digestion of dead or dying animals. The gills have been replaced by a muscular septum, capable of drawing in large food masses and the stomach has been converted into a powerful crushing gizzard, both very remarkable morphological adaptations. The style has become vestigial, and phagocytic amoebocytes no longer penetrate into the lumen of the gut. But jaws have not been acquired, and, most important of all, no extracellular protease is secreted either by the digestive diverticula or by buccal glands. In the Septibranchia the food is crushed up and the pieces so formed are ingested within the cells of the digestive diverticula. The failure to acquire the physiological adaptation, so essential to an efficient carnivore that it is even present in the much more primitive Coelenterata, represented by an extracellular protease, is probably the main reason why the Septibranchia consist of only three genera and that these are confined largely to abyssal and deep waters where there is little competition.

The final stage in the specialization of the feeding and digestive mechanisms is represented in the parasitic groups, Trematoda and Cestoda, in the second of which both disappear. Further evolution then becomes impossible.

It is notable that of these specialized feeders only one group, the Arachnida, exists on land where it possibly evolved in the first place.

VII. SUMMARY

1. Digestion in the primitive animals must have been intracellular, as it remains in the Protozoa and in the Porifera. It has persisted, to a greater or less extent, in a number of Metazoa. These may be divided into two groups: (1) those which are primitive in structure, *e.g.* Coelenterata, Ctenophora, most Turbellaria, and *Limulus*; and (2) those which are more highly evolved but have retained intracellular digestion in correlation with their mode of feeding, *e.g.* Brachiopoda, Rotifera, Tardigrada, Pyncogonida, Arachnida (other than *Limulus*) and the majority of Mollusca excluding the Cephalopoda. These animals either feed on finely divided food (collected by ciliary mechanisms or scraped by a radula) or on fluid or semi-fluid food which is sucked in.

2. In certain cases, notably the Lamellibranchia, but also in the Echinodermata, intracellular digestion is assisted or exclusively carried out by wandering phagocytic blood cells.

3. Extracellular digestion, originally developed with the increased size of available food as an aid to intracellular digestion, has completely replaced the more primitive form of digestion in certain rhabdocoel Turbellaria (probably), Polyzoa,

Annelida, Myriapoda, Crustacea, Insecta, Cephalopoda and Chordata. This mode of digestion results in the reduction of the ingestive region of the gut and enables digestion, and the removal of indigestible material, to be hastened. The resultant increase in the rate of metabolism has had profound effects on the evolution of the Metazoa.

4. The appearance of extracellular digestion has been accompanied by changes in the structure and physiology of the gut. Distinct regions have been specialized for (1) the reception of food, (2) its conduction and storage, (3) digestion and internal trituration, (4) absorption, and (5) conduction and formation of faeces.

5. There is a definite correlation between the food of any animal and the nature and relative strengths of its digestive enzymes. Certain animals have acquired specific enzymes which enable them to exploit additional sources of food, the most important of such enzymes being cellulase and chitinase.

6. There is a periodicity of secretion in the digestive glands of many Metazoa, *e.g.* Gastropoda and Crustacea. In the Lamellibranchia and in style-bearing Gastropoda, the style constitutes an ideal mechanism for the continuous liberation of small quantities of enzyme (amylase).

7. The pH of the gut is controlled in various ways in different phyla. In ciliary-feeding animals this may be of importance not only in securing the optimum conditions for the action of extracellular enzymes but also by its influence on the viscosity of the mucus with which the food is entangled.

8. There is evidence that the time taken for passage of food through the gut at any normal temperature corresponds to the period which is optimal for enzymatic action at that particular temperature.

9. The most successful groups of animals are (1) those which possess feeding and digestive mechanisms capable of utilizing, as a result of morphological and physiological adaptations, many types of food, *e.g.* Annelida, Crustacea, Insecta, Gastropoda and Vertebrata, and (2) those in which one type of food is collected and digested with great efficiency, *e.g.* Coelenterata, Turbellaria, Arachnida, and Cephalopoda (carnivorous); Brachiopoda, Lamellibranchia, and Tunicata (ciliary feeders); Trematoda and Cestoda (parasites). Of these, the first have been by far the more successful, owing to their capacity for exploiting new sources of food, in the invasion of new habitats.

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ADDENDUM

Since this article went to press the following papers have been published :

- FOX, D. (1936). *Bull. Scripps Instn Oceanogr. tech.*, 4, 1. (Digestion in *Mytilus*.)
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VERGLEICHENDE UNTERSUCHUNGEN ÜBER BEWEGUNGSSEHEN

VON PAUL VON SCHILLER
(Budapest)

(Received 8 February 1936)

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I. DER BEGRIFF DER VISUELL WAHRGENOMMENEN BEWEGUNG

(1) DER STANDPUNKT DES PHYSIOLOGEN UND DES PSYCHOLOGEN

Es ist eine merkwürdige Tatsache, dass, obwohl das Bewegungssehen eine der biologisch wichtigsten Orientierungsmittel ist, man in den Hand- und Lehrbüchern der Physiologie und Biologie sozusagen nichts über dieses Gebiet findet. Man findet Kapitel über Lichtreaktionen, neuerdings auch von Richtungs- und Formensehen, die nichts über visuell wahrgenommene Bewegung ausführen. Eine ausführliche Darstellung des menschlichen Bewegungssehens ist in dem physiolo-

gischen Handbuch von Bethe u. Bergmann zu finden und diese stammt von dem Psychologen Koffka (1931). Dieser Umstand ist recht bezeichnend für die Problemlage der visuell wahrgenommenen Bewegung.

Man sucht in der Biologie jeder Reaktion einen Reiz unterzuschieben, der eine Energiequelle darstellt. Diese Energiequelle veranlasst im Rezeptor eine Veränderung der Energieverhältnisse, die zur Erregung und so zur Rezeption führt. Was ist aber die Reizenergie im Falle visuell wahrgenommener Bewegung? In der Umgebung ist lediglich eine geometrische Veränderung eingetreten, und diese stellt keine physikalische Energiequelle dar, die eine relaisartige Auslösung veranlassen könnte. Die Bewegungsperzeption ist also nicht an das Auftreten einer Energie in der Umgebung gebunden, sondern lediglich auf die Umgruppierung vorhandener Energieverteilung. Diese Vorstellung passt so wenig zu der herrschenden Auffassung mechanischen Sinnesgeschehens, dass der Physiologe dieses Problemgebiet zum guten Teil dem Psychologen überlassen hat.

Der Psychologe betrachtet den Reiz nicht als eine Energie sondern als eine Situationsänderung, die der Organismus erfährt. Eine Veränderung in der Umwelt ist ein Reiz, ob dabei Energien im einfachen Sinne frei werden oder nicht. Dabei spricht der moderne Psychologe nicht mehr von selbstständigen psychischen Vorgängen, die von physiologischen unabhängig funktionieren und das Wahrnehmen den physiologischen Bedingungen entgegen beeinflussen, wie das auch bei der Behandlung von Bewegungssehen in manchen Lehrbüchern noch üblich ist (Fröbes, 1923). Psychologische Vorgänge sind eben ganz anderer Ordnung als physiologische, sie bestehen nur wenn man von ganz anderen Gesichtspunkten an die Lebenserscheinungen herantritt als der Physiologe, aber sie sind ohne zugrundeliegende physiologische Prozesse nicht denkbar. So kommt es, dass die physiologischen Theorien des Bewegungssehens meist von Psychologen stammen, die eigentlich nur die makroskopischen Leistungen des Organismus in seiner Umwelt untersuchen, diese aber ohne die mikroskopischen Vorgänge innerhalb des Organismus nicht verstehen können.

Der Psychologe ist in erster Reihe an den Menschen interessiert und greift zum tierischen Verhalten nur dort, wo ihn ein Vergleich mit dem Menschen interessiert. In diesem Sammelreferat möchte ich aber gewissermassen umgekehrt vorgehen. In Verfolgung der Frage: Was wissen wir über die Leistung Bewegungssehen im allgemeinen, sollen nur jene Fragen behandelt werden die für Vertreter der Tierwelt untersucht worden sind und ziehe dabei den Menschen nur insofern heran, als die Feststellungen die durch nur beim Menschen mögliche verbale Erlebnisanalyse (Untersuchung des "Phänomenalen") ermittelt worden sind, für das Verständnis des tierischen Bewegungssehens notwendig sind. So wird in diesem Bericht von manchen Phänomenen, die nur für den Menschen gesichert bestehen (wie z. B. von autokinetischen und anorthoskopischen Bewegungen sowie von Bewegungsnachbildern), nicht die Rede sein. Es könnte an anderer Stelle gezeigt werden, dass diese sich in das allgemeine Bild fügen, das vergleichende Untersuchungen über die biologische Leistung "Bewegungssehen" als eine Fähigkeit von Organismen bestimmter Organisationsstufe zeichnen.

Die Rahmen eines Sammelreferates werden an einigen Stellen übertreten, dort nämlich, wo die Mitteilung einiger noch nicht veröffentlichten Bearbeitungen und Überlegungen dem Verfasser zur Ergänzung des Bildes notwendig erscheinen. Das wird im Text gesondert vermerkt. Ich werde die Wahrnehmung reeller (d. h. an Lichtverschiebung auf der Retina gebundener) Bewegung und die sog. Scheinbewegung gesondert behandeln, und in jeder dieser Gruppen die Leistungen der beiden Haupttypen von Lichtsinnesorganen, das Linsenauge und das Facettenauge gesondert betrachten, um zum Schluss die Theorien zu erörtern, die bei den verschiedenen Organtypen und verschiedenen Reizverhältnissen gewonnenen Ergebnisse zu vereinigen versuchen.

(2) SENSATION UND PERZEPTION

Diejenige hypothetische Funktion, die eine Zuordnung der Verhaltensvariation zur Variation in der Umwelt der Organismen besorgt, nennt sich *Rezeption*. Wir schliessen auf das Vorhandensein von Rezeption, wenn das Verhalten eines Organismus eine Variabilität zeigt, die parallel geht mit einer Variation in der Umgebung. Eine wichtige Unterscheidung der Rezeptionsvorgänge ist hier einzuführen. Wird eine bestimmte Verhaltensweise durch eine Reihe verschiedener Umweltvariationen immer in gleicher Weise hervorgerufen, so sprechen wir von dem Spezialfall *Sensation*, z. B. wenn ein Tier auf Belichtung sich zusammenzieht, gleich aus welcher Richtung die Belichtung kommt und—wenn die Reaktion überhaupt zustandekommt—auch von der Intensität unabhängig. Wir sprechen aber vom Spezialfall *Perzeption*, wenn einer Reizmannigfaltigkeit eine Antwortmannigfaltigkeit zugeordnet ist. Insbesondere ist eine Zuordnung verschiedener, entsprechender Reaktionen zu der zeitlichen und räumlichen Lage der Reize für die Perzeption kennzeichnend und wir nennen die Perzeption umso feiner, je kleineren Änderungen in der Umgebung eine Reaktionsänderung entspricht.

Ist ein Bereich der Umgebung in räumlichem und zeitlichem Wechsel, und entspricht dem eine eindeutig zugeordnete Reaktionsveränderung, so ist Bewegungsperzeption vorhanden. Diese ist von den Bewegungssensationen zu trennen, die eine einzelne Reaktionsart auf jede Bewegungsart darstellen, z. B. das Einziehen des Rankenfusses von *Balanus*, die bei vorbeiziehender Bewegung jedweder Richtung und in weiten Grenzen von der Geschwindigkeit derselben unabhängig erfolgt (Buddenbrock, 1930); ist die Geschwindigkeit zu gross oder zu klein, so erfolgt überhaupt keine Reaktion. Die Reaktionen von dem Typus Alles-oder-nichts sind bezeichnend für die Sensation. So ist also Bewegungsperzeption nicht gleichbedeutend mit Reaktion auf Bewegung, sondern ein Spezialfall von diesem.

Die Bewegungssensation ermöglicht keine der jeweiligen Bewegungsphase zugeordnete Beantwortung, sondern bloss eine auf jedwede Bewegung gleichgeartete Reaktion. Demgegenüber ermöglicht die Bewegungsperzeption das Ausführen entsprechender Handlungen dem bewegten oder sonstwie wechselnden Umweltbereich gegenüber indem die Reaktion je nach Geschwindigkeit und Richtung dieses Wechsels entsprechend erfolgt.

Von *visuell wahrgenommener Bewegung* (Bewegungssehen) ist in dem Falle die

Rede, wenn einem optischen Wechsel in der Umgebung, bei alleiniger Vermittlung des Lichtes eine Reaktion entspricht, die je nach dem Stand dieses Wechsels spezifisch gerichtet ist. Das Bewegungssehen ermöglicht Fernreaktionen, d. h. eine Zuordnung des Verhaltens zu Umgebungsänderungen, die weder mechanisch noch chemisch mit dem Organismus in Berührung kommen, sondern die Lebensmedien der Organismen lediglich auf dem Lichtwege durchdringen. So ist es z. B. der Forelle möglich, aus dem Wasser die darüber in der Luft herumfliegende Mücke zu verfolgen und zielgerecht nach ihr zu schnappen. Das Bewegungssehen ist also eine Funktion der visuellen Orientierung.

II. DAS SEHEN REELLER BEWEGUNG

Das Wild flieht vor dem bewegten Menschen, obwohl es an dem ruhenden ungestört vorbeigeht. Die männliche Schildkröte bemerkt das Weibchen nur wenn es vorbeischwimmt, das unbewegte bleibt ihr unbemerkt (Courtis, 1907). Frösche, Salamander und Eidechsen schnappen im allgemeinen nur nach bewegtem Futter (Edinger, 1908) und Kahmann (1934) zeigt, dass Schlangen nur bewegte Beute wahrnehmen. Viele Vertebraten steuern ihre Verteidigung, die Ernährung und das Liebesleben nach bewegten Signalen. Über das Sehen der Avertebraten liegen nur Angaben vor, die sich auf höchstorganisierte Spezies beziehen, auf Kephалopoden und auf Arthropoden. So berichtet Buddenbrock (1928) von einem ruhenden Octopus, der einen in der Nähe sich bewegenden Gegenstand mit den Augen verfolgt und in gefährlicher Situation aus dem Feinde zugekehrtem Trichter Wasser ausspritzt, so dass er dadurch in entgegengesetzter Richtung wegschwimmt.

Doch lässt sich in solchen Schilderungen meist nicht klar durchschauen, wiefern solches Verhalten optisch gesteuert ist und ob nicht chemische und mechanische (z. B. Strömungs-) Sinne mitbeteiligt sind. Bei der Behandlung der Frage, ob ein gerichtetes Verhalten in bezug auf Bewegung in der Umwelt optisch gesteuert ist, muss eben für den Ausschluss nicht-optischer Reize Sorge getragen werden, was nur unter experimentellen Bedingungen geschehen kann.

(1) DIE AVERTEBRATEN

(a) Dofleins Beobachtungen an Dekapoden

Experimentelle Untersuchungen über das Bewegungssehen Avertebrater sind nur an Arthropoden angestellt worden. Doflein (1910) beobachtete, dass verschiedene dekapode Krebse, wie die Leanderarten, die Paguriden und vor allem *Galathea* mit ihren Antennen, die chemische Sinnesorgane tragen, bewegten leuchtenden Gegenständen folgen. Diese Reaktion tritt auch dann ein, wenn die bewegten Objekte sich ausserhalb des Aquariums befinden. So ist klar, dass weder Wasserbewegung, noch irgendwelche chemische Reize für die Reaktion verantwortlich sind. Für *Galathea squamifera* und *G. strigosa* besteht diese Reaktion bis auf eine Entfernung von 1.5 m. des Reizobjekts vom Tier, entsprechende Grösse des Reizobjekts vorausgesetzt. Diese sog. Signalreaktion wird auch von einem kleinen

Objekt von 1 cm.² ausgelöst, wenn dieses sich nicht entfernter als 10 cm. vom Tier bewegt. Die Reaktion soll dazu dienen, den gesehenen Reiz auf seine chemische Eigenschaften zu prüfen. Ich konnte mich bei Nachprüfung dieser Experimente (im Aquarium zu Rovigno, s. w. u. III, (2)) überzeugen, dass *Galathea squamifera* das absinkende Futter mit der ihm folgenden Antenne abtastet bevor sie es mit der Schere packt. Die mitgeteilten Abbildungen (Fig. 1) sind nach Filmaufnahmen gezeichnet, die ich von der Signalreaktion von *Galathea* gemacht habe.

(b) Die optomotorische Reaktion

Diese Untersuchungen Dofleins blieben lange Zeit ohne Nachfolger. Erst viele Jahre später wurde das Thema "Bewegungssehen der Arthropoden" wieder aufgenommen, in einem ganz anderen Zusammenhang. Schlieper beobachtet 1926 den dekapoden Krebs *Hippolyte* in einem ruhenden Behälter inmitten eines gedrehten Zylinders, dessen Innenwand mit schwarz-weißen Vertikalstreifen gemustert ist, um die dabei entstehende optomotorische Reaktionen zur Erforschung des Farbensinnes zu benützen. Die optomotorischen Reaktionen bestehen in einem Mit- (oder Gegen-) bewegen einzelner Körperteile oder des ganzen Körpers in bezug auf die Musterbewegungsrichtung. Sie ist von Loeb und anderen vor langer Zeit untersucht worden und soll eine kompensatorische Reaktion auf Gedrehtwerden darstellen, die auch bei Ausschaltung rheo- und geotaktischer Orientierung, phototaktisch erfolgen soll (Rádl, 1902). Nach Schlieper (1927) ist die optomotorische Reaktion, die nicht nur an verschiedenen Dekapoden, sondern auch an Schmetterlingen (*Epinephele*, *Vanessa*), an der Heuschrecke *Mantis*, an *Coccinelliden*, an *Apis* und auch an Wirbeltieren, wie Fischen und Eidechsen und sogar am Menschen (vgl. III, (1)) feststellbar ist, aus der Summierung kleiner Korrekturbewegungen zu verstehen, die auf das Festhalten der Reizverteilung auf demselben Retinastellen gerichtet sind, also aus Summierung menotaktischer Bewegungen (Kühn, 1929), die sich aus Reaktionen auf einzelne unterschiedene Lageveränderungen ergeben.

Dieser Auffassung gegenüber weist Gaffron (1934) nach, dass Aeschnalarven, die im Drehzylinder mitschwimmen, auf das verdeckte Verrücken eines Musters nicht mit Lagekorrektur antworten; es ist also die gesehene Bewegung nötig um die Reaktion hervorzurufen. Auch die Hausfliege reagiert immer im Richtungssinne der Umfeldrehung. Die Fliegen drehen sich, in welcher Stellung oder Lage sie auch immer sich gerade befinden, um diejenige ihrer Körperachsen, die mit der Achse der Bewegung in der Umgebung zusammenfällt, wenn sich dabei auch ganz verschiedene Körperbewegungen ergeben. Im engen Glaszylinder kriechen sie gerade aufwärts in einer der Musterdrehrichtung entsprechenden schiefen Haltung, auf einem Glasstab dagegen kriechen sie in Spiralen herum, also beide Male um die Achse der Umfeldbewegung. Die Bewegungsrichtung wird also unmittelbar unterschieden. Vergleichende Untersuchungen mit *Coccinelliden* ergaben, dass diese schon bei der Verrückung eines einzigen Streifens Lagekorrektur vornehmen und dass die Reaktion ausbleibt, wenn das Feld überall mit dem Muster erfüllt ist, obwohl bei *Aeschna* und der Fliege die reiche Gliederung gerade begünstigend wirkt. Bei der Drehung zweier benachbarter Streifen sind bei den höheren Insekten

keine, bei Coccinellen deutliche ruckartige Körperbewegungen vorhanden. So gewinnt man den Eindruck, dass diese niederen Käfer nicht auf ein bewegtes Feld

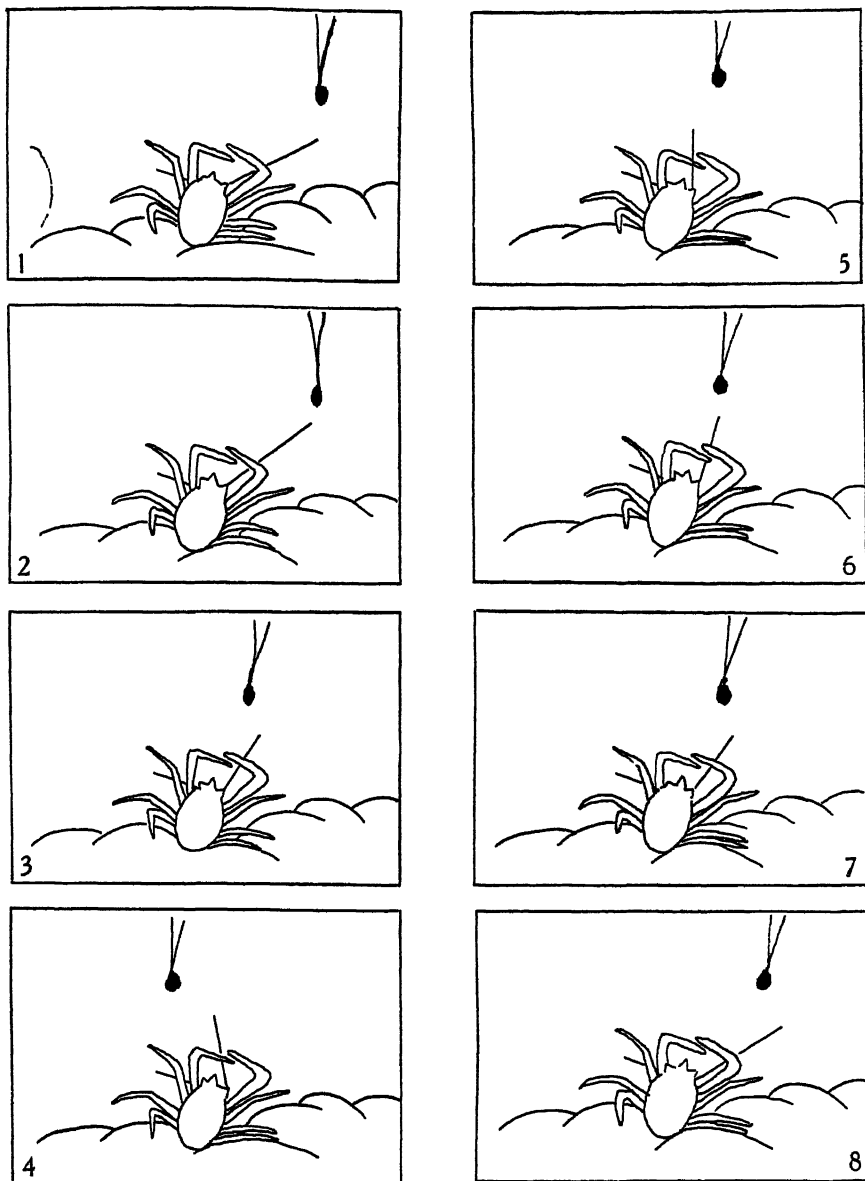


Fig. 1. *Galathea squamifera* folgt mit der rechten Antenne einem Signal. (Nach Filmaufnahmen des Verfassers gezeichnet.)

reagieren, wie *Musca* und *Aeschna*, sondern auf einzelne Lageveränderungen, also menotaktisch. Demgegenüber zeigen die Fische im Drehzylinder ausgesprochene Bewegungsreaktionen, indem sie nur dann gut mitgehen, wenn das Feld allseitig

gegliedert ist und sie korrigieren nie bei einmaligem Verrücken von einigen Streifen, wie die Coccinellen das tun (vgl. III (1)).

(c) *Wolfs Versuche an Apis*

Für das Bewegungssehen in unserem Sinne gewinnen die optomotorischen Reaktionen erst recht an Interesse, wenn sie zur biologischen Orientierung der Tiere beitragen. Das ist der Fall nach ausgedehnten Versuchen von Wolf und Mitarbeitern an der Honigbiene.

Wird unter der Kriechplatte, auf der sich entflügelte Bienen befinden, ein Streifenmuster bewegt, so marschieren die Bienen in der Bewegung entgegengesetzter Richtung. Es wird festgestellt bei welcher Dichte der Bemusterung die Reaktion noch auftritt. Diese Dichte entspricht den Grenzen des optischen Auflösungsvermögens des Bienenauges (Sehschärfe) (Hecht & Wolf, 1929). Bei variierter relativer Streifenhelligkeit kann auch die Intensitätsunterscheidung untersucht werden. Die Sehschärfe beträgt $1/100$, die Unterscheidungsempfindlichkeit $1/25$ des Menschauges (Wolf, 1935).

Bei der Untersuchung der spontanen Reaktionen auf verschiedene gestreifte Muster ergibt sich, dass die Bienen das dichtere von zwei Mustern bevorzugen (Zerrahn, 1934). Werden statt zwei, mehrere Muster dargeboten und zwar gedrehte Scheiben, die eine verschiedene Zahl von schwarzen und weissen Sektoren tragen, so ergibt sich, dass die Bienen in grösserer Schar diejenigen Scheiben besuchen, die einen stärkeren Reizwechsel darbieten (Wolf, 1934). Zählt man die Verteilung der Bienen auf die verschieden frequenten Reizwechsel darbietenden Scheiben, so ergibt sich die unten stehende Tabelle. Um dem Einwand zu entgehen, dass dabei die verschiedene Gesamthelligkeit der Scheiben mitwirken könnte, hat Wolf auch solche Scheiben konstruiert, die bei verschiedener Dichte der Sektoren konstante Gesamthelligkeit besaßen. Dabei blieben die Ergebnisse im Prinzip unverändert. Dasselbe gilt von Versuchen, die im Freien ausgeführt worden sind. Das besagt also, dass die Stärke der Reizabwechslung ein Orientierungsmittel der Bienen ist, in dem Sinne, dass die Bienen umso mehr angezogen werden, je lebhafter die Bewegung im Reizfeld ist (Flimmertaxis).

Tabelle I

Reizabwechslung pro sec.	3.1	6.2	9.3	12.4	15.5
Bienenzahl	10	48	72	98	135
Bienenzahl bei kompensierter Helligkeit der Scheiben	19	32	52	65	95
Bienenzahl im Freilichtversuch	10	24	32	42	60

Die Bewegung dient den Bienen als Merkmal, was aus dem folgenden Versuch noch klarer hervortritt (Wolf, 1934). Werden zwei Strahlenfiguren mit 6 weissen und 6 schwarzen Sektoren zur Wahl dargeboten, von denen die eine ruht, die andere aber gedreht wird, so zeigen die Bienen eine Bevorzugung der gedrehten, wenn diese schneller als $1/2$ Umdrehung pro sec. gedreht wird und nicht zu schnell. Wie die anderen behandelten optomotorischen Reaktionen, so hören auch diese auf,

wenn die Bewegung zu rasch wird, da infolge von Verschmelzung keine Innengliederung mehr gegeben ist (vgl. II, (2)). Bei geeigneter Geschwindigkeit ist die Bevorzugung der gedrehten Scheibe 3·8 : 1, bei einer anderen Figur sogar 5·7 : 1. Zerrahn (1934) zeigt, dass bei der Wahl ruhender Figuren die Wahlanzahl den Konturlängen proportional ist. Dabei kommt es auf die Bewegungsperzeption an, da die Figuren im Flug gesehen werden, wobei die Musterung an dem Auge vorbeigezogen, also in relativer Bewegung gesehen wird. "Was wir als die Wahl der Figuren bezeichnen, ist eine Zwangsreaktion, erzeugt durch die Stärke der Flimmerwirkung der Figuren" (Wolf, 1935). Flimmern ist der Eindruck periodischen Lichtwechsels, einer Bewegung die in keine bestimmte Richtung fortschreitet und entsteht als Zwischenstadium zwischen Bewegung und Verschmelzung (s. II, (2)). Für die phototaktische Orientierung der Biene ist es gleichgültig ob eine grosse Anzahl von Rezeptoren mit geringer Flimmerfrequenz gereizt wird oder eine kleinere Anzahl entsprechend öfter in der Zeiteinheit. Eine kleine stark flimmernde und eine grosse wenig flimmernde Fläche werden gleich häufig besucht, wenn die Reizabwechslungszahlen in beiden Fällen gleich sind. Nach diesen Gesetzen soll sich die Anziehung der Bienen an die blühenden Obstbäume beim Überflug ergeben.

Als obere Grenze des Flimmersehens ist eine Abwechslung von 52·6 Reizen pro sec. festgestellt worden, die auch bei gesteigerter Helligkeit nicht mehr überboten wird (Wolf, 1933). Dieser Wert stimmt ganz gut überein mit einem von Sälzle (1933) gefundenen Wert. Sälzle untersuchte die Libellenlarve *Aeschna*, die ihre Fangmaske nach bewegten Gegenständen vorschleudert. Diese Reaktion war bei einer Geschwindigkeit von 59·7 Umdrehungen pro sec. noch vorhanden. Als untere Grenze ist 0·9–2·8 Umdrehungen festgestellt worden, je nach der Lichtstärke. Zur Erklärung der Verschmelzung greift Sälzle auf das Phänomen der Refraktärphase, eine reversible Desensibilisierung des photosensitiven Materials, zurück. Vergleiche das in II, (2), über das zeitliche Auflösungsvermögen gesagte.

(d) Die Hertz'schen Versuche an *Apis* und *Musca*

Wenn diese Arbeiten der Wolf'schen Schule über das Bewegungssehen sozusagen nur nebenbei unterrichten, weil sie die Funktionen der Sinnesorgane an Hand äusserer Bewegungsvorgänge untersuchen und nicht die spezifisch gerichtete Reaktion des Tieres auf Bewegung, so stammen die neuesten Arbeiten von Hertz und ihrer Mitarbeiterin Gaffron aus einer weniger physikalischen und physiologischen als vielmehr psychologischen und biologischen Fragestellung. Worauf begründet sich der Unterschied bewegter und nicht-bewegter Objekte bei dem Tier, das sich bewegend diese Unterscheidung vornimmt, wobei doch sein Auge von immer wechselnden Reizen getroffen wird?

Fliegen unterscheiden objektive Bewegung von der durch die Eigendrehung verursachten Bildverschiebung, wie folgender Versuch von Hertz (1934 b) lehrt (vgl. Fig. 2).

Wird in einem doppelten Drehzylinder das gleiche Muster in der unteren und der oberen Sehfeldhälfte gezeigt und nur die eine Hälfte bewegt, so geht die Fliege

in der Richtung der bewegten Hälfte mit, gleich ob die bewegte Hälfte die obere oder untere ist und ob sie von den beiden bemusterten Innenwänden die nähere oder die fernere ist. Mann muss bedenken, dass dabei die ruhende Hälfte in entgegengesetzter Richtung am Auge vorbeizieht, was also besagt, dass die Fliege dieses Vorbeiziehen von der objektiven Bewegung des Musters unterscheidet. Diese Reaktion bleibt erhalten auch wenn das ruhende Sehfeldteil die grössere Hälfte der Innenwand ausmacht. Es gehört ein achtfaches Überwiegen des ruhenden Teils über dem bewegten zum Aufheben der optomotorischen Reaktion. Nach Hertz (1934 b) kann sich diese Unterscheidung der objektiven Bewegung von derjenigen Bildverschiebung, die als Folge der Eigenbewegung der Fliege entsteht, nicht aus den optischen Vorgängen allein erklären. Es ist das Verhältnis der optischen und der motorischen Vorgänge auf das sich diese Reaktion gründet. Sind keine motorischen Vorgänge vorhanden, wie etwa bei *passiver Bewegung* der Tiere, so entsteht eine optomotorische Reaktion auf die dabei auftretende Bildverschiebung (Alverdes, 1924; Baldus, 1927; Gaffron, 1934) die, wie eben gesagt, nicht auftritt wenn diese Bildverschiebung durch *aktive Eigenbewegung* bedingt ist.

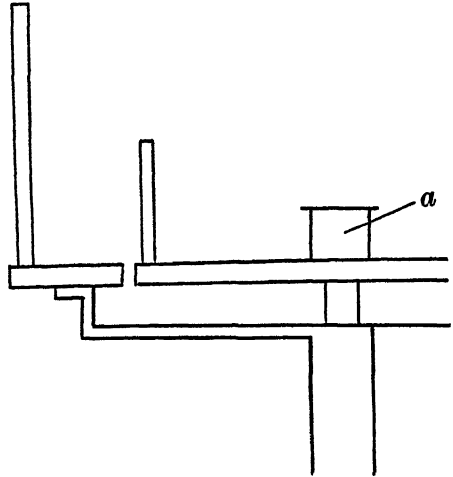


Fig. 2. Schema der Anordnung für optomotorische Reaktionen an *Musca domestica*. Der doppelte Drehtisch von Gaffron und Hertz lässt mannigfache Variationen zu. a, Fliegenbehälter.

(e) *Bewegungssehen und Formsehen*

Von Bewegungssehen nach unserer Definition kann erst gesprochen werden, wenn ein Unterscheiden des Bewegten vom Unbewegten gelingt, also Inhomogenitäten im Raum ihrer Gliederung nach wahrgenommen werden, was bildentwerfende Optik voraussetzt. Das ist aber auch die Voraussetzung für das Formsehen. Formsehen besteht, wenn verschiedene Gliederung gleichgrosser Raumabschnitte unterschieden wird. Wolf (1935) behauptet nun, dass bei Insekten kein echtes Formsehen in diesem Sinne vorliegt, weil sie nur den vom Konturreichtum bedingten Reizwechsel wahrnehmen, also die Quantität der Gliederung, nicht aber das Zueinander der Konturen, also die Qualität der Gliederung. Soll also echtes Formsehen der Bewegungswahrnehmung gegenüber nachgewiesen werden, so muss man zeigen, dass quantitativ gleichgegliederte, aber ihrem Zueinander nach anders geartete Figuren unterschieden werden.

Das wird von Hertz (1934, b, c) in der Tat nachgewiesen. Nicht der blosse Reichtum an Konturen (der proportional mit der Innengliederung wächst) ist entscheidend bei der Wahl, sondern auch das Richtungszueinander und die Unstetigkeit der Konturen. Ist die konturärmere Figur weniger stetig in ihrer Gliederung

derung, so kann sie dem reicher und stetiger gegliederten gegenüber bevorzugt sein, was beweist, dass keine Flimmertaxis die Biene lenkt. Solche Versuche lassen sich bei geeigneter Wahl des Figurenpaars auch so ausführen, dass in reziproker Dressur einmal die eine, ein anderes mal die andere Figur zum positiven Dressurmerkmal wird.

Nicht nur die Reaktion auf ruhende gegliederte Formen, sondern auch die Reaktion auf bewegte Bilder ist anpassungsfähig. Bietet man eine ruhende und eine bewegte Figur, so kann man die Bienen dressieren, die bewegte zu vermeiden und die ruhende aufzusuchen. Der Wolf'sche Versuch besagt also nicht, dass Bewegung eine Zwangsreaktion veranlasst sondern nur soviel, dass bewegte Figuren eindringlicher wirken und daher bevorzugt werden. Wird eine Figur bewegt, so wirkt sie abgehobener und eindringlicher als in Ruhe (Hertz, 1933). Wird eine mit Hilfe von Bewegung abgehobene Figur wahrgenommen, die sonst nicht hätte gesehen werden können, z. B. wenn man einen im Sand liegenden Rochen im Aquarium erst bemerkt, wenn dieser sich bewegt, so sprechen wir von keiner Bewegungswahrnehmung in dem hier verstandenen Sinne, sondern von Formsehen. Also ist der Wolf'sche Satz: figurale Qualitäten werden als Bewegung wahrgenommen (also von Bewegung nicht unterscheidbar) in dieser Form nicht zu verteidigen.

Die sichtbare Bewegung ist also nicht lediglich erregungssteigernd für den betreffenden Bereich, sondern eine von anderen figuralen Eigenschaften unterscheidbare Eigenschaft, wenn nur der Bewegungscharakter deutlich, d. h. Richtungsbestimmtheit vorhanden ist. Die fortschreitende Bewegung eines Kreises wird vom gleichen ruhenden Kreis unterschieden, jedoch nur als negatives Dressurmerkmal. Als positives Merkmal aber konnte die Bewegung in diesen Versuchen von Hertz (1934 c) nicht dienen. Im Gegensatz zu jagenden Tieren, denen Bewegung als positives Futtersignal dient, ist die Ruhe der Blüten für die Bienen ein positives Futtersignal, das sich nicht einmal in der Dressur ändert. Bei der Biene ist nun der physiologische Grund der Unterscheidung der fortschreitend bewegten von ruhenden Figuren darin zu suchen, dass die von der ruhenden Figur veranlassten Lichtverschiebungen mit dem Landungsmanöver langsamer werden (bis zum Stillstand), wohingegen die objektiv bewegte Figuren auch bei der Landung weiteren Reizwechsel bedingen. Weiteres über Bewegungssehen der Arthropoden, s. III, (2).

(2) DIE VERTEBRATEN UND DER MENSCH

(a) *Schwellenfragen*

Wie alle Sinneserscheinungen, so sind die Bewegungswahrnehmungen an Schwellenwerte gebunden.

Am Menschen ausgeführte Versuche unterrichten über die Grenzen der Bewegungswahrnehmung. Werden Objekte zu schnell bewegt, so erlebt man keine Bewegung sondern eine unbestimmte Veränderung, oder wenn die Bewegung periodisch ist, ein homogen erfülltes Feld. Sind bei geringer Geschwindigkeit

eines Objekts auch ruhende Objekte vorhanden, so genügt eine Bewegung von 1–2 Min. pro sec. zum Bewegungssehen (Aubert, 1886), im Dunkeln sind 14–28 Min. nötig und für grössere Objekte sogar 40 Min. (Bourdon, 1902 und früher). Dementsprechend erscheinen grössere Objekte im allgemeinen langsamer bewegt als kleinere, bei gleicher physikalischer Geschwindigkeit. Nach Brown (1931 c) ist die untere Bewegungsschwelle (auch die kinematoskopische und die Verschmelzungsschwelle, s. III, (1)) je nach der Grösse und Struktur des Feldes, das als Hintergrund zur Bewegung dient, verschieden. Auch ist die wahrgenommene Geschwindigkeit verschieden je nach der Struktur des Feldes, der Grösse der bewegten Figur, der Richtung der Bewegung usw. (Brown, 1931 a). So erscheint z. B. ein Balken der in der Richtung seiner grösseren Ausdehnung sich bewegt mit grösserer Geschwindigkeit als einer der mit physikalisch gleicher Geschwindigkeit in Richtung der kleineren Ausdehnung sich bewegt. Durch Vergleich sind auch quantitative Feststellungen gemacht worden. Verhalten sich zwei Felder der Grösse nach wie 2 : 1, so erscheint eine Bewegung von 10 cm. pro sec. in 2 einer in 1 gleich schnell, die 5·20 cm. pro sec. bemisst. Mit der wahrgenommenen Geschwindigkeit ändert sich auch die wahrgenommene Dauer und der wahrgenommene Weg (Brown, 1931 b). Auch für die Unterschiedsschwelle zweier Bewegungen ist die Feldstruktur und figurale Beschaffenheit des bewegten Gegenstandes mitbestimmend, sie ist z. B. grösser wenn sich die grösser erscheinende Müller-Lyer Figur, als wenn sich die kleiner erscheinende bewegt (Brown, 1932). (Vgl. Fig. 5.) Die Werte der Unterscheidungsempfindlichkeit sind für verschiedene Netzhautpartien verschieden.

Schwellenbestimmungen wurden auch an Ratten und an Hühnern vorgenommen. Hawley und Munn (1933) liessen im T-Apparat ein bewegtes von einem ruhenden Streifenmuster unterscheiden. Das ist den Ratten ohne weiteres gelungen, wenn die Geschwindigkeit 25 cm. pro sec. war und bei fortgesetzter Übung ist die Wahl noch zu 78 % Sicherheit gelungen wenn die Bewegung nur 4 cm. pro sec. war. Pattie und Stavsky (1932) nahmen zwei bewegte Trommeln, wobei die schneller bewegte von den Hühnern gewählt werden sollte. Nach Erfolg konnte man die absoluten Geschwindigkeiten in weiten Grenzen variieren ohne die Reaktion zu stören. Es ergab sich also eine sogenannte Strukturfunktion d. h. eine Reaktion die nicht auf einen bestimmten Einzelreiz, sondern auf ein bestimmtes Verhältnis von Reizen sich bezieht, wie sie Coburn (1914) und Köhler an Vögeln und höheren Vertebraten nachgewiesen haben und Perkins (1931) sie auch an Fischen für relative Helligkeitspaare feststellte.

Über die Lokalisation des Bewegungssehens im Z.N.S. unterrichten einige Beobachtungen über grosshirngeschädigte Tiere und Menschen. Schrader (1889) fand, dass das Bewegungssehen seiner grosshirnlosen Falken intakt geblieben war. Ähnliche Versuche hat van Essen (1934) angestellt. Bewegt man ein 10–30 cm. entferntes grosses Stück Karton vor den Augen einer enthirnten Ente, so folgt der Kopf und Hals und später das ganze Tier dem Karton. Bei Annäherung des Kartons zeigt das Tier Ausweichreaktion. Ein sensomotorischer Apparat sorgt für das Scharferhalten des Netzhautbildes, wobei der Intensitätsunterschied naher und ferner Reize auslösend ist. Diese Hypothese ist aber nicht geprüft worden. Jeden-

falls kann soviel festgestellt werden, dass Bewegungssehen nicht an einen Kortex gebunden ist, da es einerseits auch bei Avertebraten vorliegt, andererseits aber auch bei dezerebrierten Vertebraten vorhanden bleibt. Über Beobachtungen an Menschen, sowie weiteres über die Lokalisation siehe weiter unten III, (1).

(b) *Verschmelzung und der Uexküll'sche Moment*

Zu den Schwellenfragen des Bewegungssehens gehören die Erscheinungen des Flimmerns und Verschmelzens, die an einer rotierenden Scheibe mit schwarzen und weissen Sektoren zu beobachten sind. Wird eine Sektorenscheibe rotiert, so sieht man bei langsamer Umdrehung getrennte Sektoren, die sich bei rascherer Rotation vervielfachen, bis bei noch schnellerer Rotation ein Flackern, Flimmern, Zittern eintritt, das nunmehr als wahrgenommene Veränderung, ohne Richtungsangabe gilt. Bei noch schnellerem Umdrehen verschwindet auch das Zittern und man nimmt eine homogene Scheibe wahr. Beim Verschmelzen ist trotz periodischer Lichtverschiebung keine phänomenale Bewegung vorhanden.

Die Schwellenwerte des Übergangs von der Bewegung ins Flimmern und vom Flimmern ins Verschmelzen sind von vielen Faktoren abhängig, wie z. B. von der Adaptation (Lythgoe, 1929), von den Farbverhältnissen und von der Beleuchtung der Umgebung (Hecht, 1933), von der Verteilung des Flimmerns auf beide Augen (Vernon, 1934) und von heterosensoriellen Eindrücken (Schiller, 1935), um nur die neueste Literatur zu nennen. Infolge der Rezeptionsträgheit der Netzhaut, oder wie man auch sagen kann, wegen der Beschränktheit des zeitlichen Auflösungsvermögens des optischen Organs (siehe weiter unten) ist ein rascher Wechsel nicht mehr getrennt wahrzunehmen sondern es entsteht der Eindruck der Gleichzeitigkeit. Sieht man im Dunkeln einen leuchtenden Körper sich rasch bewegen, so ist die Bewegungsbahn in einer bestimmten Ausdehnung gleichzeitig sichtbar.

Die Werte für die Verschmelzung sind je nach Umständen sehr verschieden, sie variieren beim Menschen zwischen $1/20$ und $1/75$ sec. für den Teilreiz. Auf Grund von vergleichenden Untersuchungen am Menschen, am Kampffisch und an der Weinbergschnecke hat Brecher (1932) nachgewiesen, dass dieser Wert, der sog. Moment (Uexküll) für die einzelnen Tiergattungen charakteristisch ist. Er sei für den Kampffisch etwa halb so lang wie für den Menschen, für die Weinbergschnecke dagegen viermal so lang. Brecher behauptet auch dass dieser Wert für alle Sinne gleich sei. Diese Behauptung ist aber von Skramlik (1934) auf Grund älterer Experimente für den Menschen widerlegt worden, indem er zeigte, dass diese Werte für die verschiedenen Sinne in weiten Grenzen variieren und nur unter zufälligen Bedingungen zum Teil zusammenfallen können. Diese Widerlegung bestreitet natürlich nicht, dass es auf jedem Sinnesgebiet Verschmelzung und ihre Vorstadien gibt, und dass es eine allgemeine Eigenschaft der Rezeptionsorgane ist, die wir als zeitliches und räumliches Auflösen bezeichnen (siehe weiter unten).

Wird dem siamesischen Kampffische *Betta splendens* sein Spiegelbild gezeigt, so fährt er auf es los wie auf einen Gefährten. Wird zwischen Spiegel und Tier eine Scheibe mit Ausschnitt rotiert (sog. Episkotister), so ist die untere Grenze der Geschwindigkeit festzustellen bei der die sukzessiven Bilder verschmelzen: da zeigt

das Tier die Kampfreaktion. Mit Hilfe dieser Methode hat Lissmann (1933) (und auch Brecher) die Verschmelzungsschwelle festgestellt. Dieser Wert liegt beim Kampffische viel höher als beim Menschen, so dass die einzeln noch getrennt wahrnehmbaren Reize von kleinerer Dauer sind als beim Menschen. Dieser Zeit entspricht der Moment, den die Schule von Uexküll als einen die Umwelt des Organismus mitbestimmenden Faktor betrachtet. Nach Lissmanns Berechnungen erscheinen dem Kampffisch die Bewegungen etwa halb so schnell wie dem Menschen (nach Beniuc (1933) noch langsamer), weil sein Moment sehr kurz ist, also eine gegebene Bewegung erfüllt bei ihm viel mehr Momente als beim Menschen. Im Moment sind keine zwei Eindrücke mehr zu unterscheiden, seine Ausfüllung ist homogen.

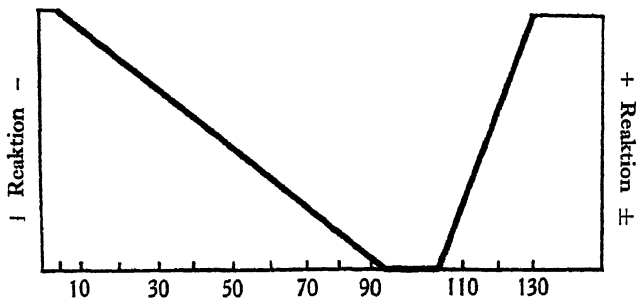


Fig. 3. Die Kurve zeigt das Verhältnis der negativen und positiven Reaktionen von *Betta splendens* auf eine rotierende Sektorenscheibe. Bei Umdrehung von 5-90 pro sec. wird negativ, bei 105-130 pro sec. positiv reagiert, sonst schwankend. (Nach Beniuc.)

Beniuc (1933) dressierte diesen Fisch auf eine langsam rotierte Sektorenscheibe. Die so erworbene Reaktion wurde bei schnellerem Rotieren beibehalten, bis 90 Reizwechsel pro sec. Bei grösserer Geschwindigkeit war die Reaktion zögernd, bis über 120 Reizwechsel diejenige Reaktion auftrat, die in bezug auf ruhende Scheiben erworben worden war. Dabei diente als Merkmal die Bewegung. Denn wählte Beniuc eine graue Scheibe mit vielen schmalen Sektoren, etwa wie der Mensch die gedrehte Scheibe in einem bestimmten Drehstadium sieht, so antwortete der Fisch mit der für Ruhescheiben erlernten Reaktion. Das beweist, dass die Reaktion wirklich an Bewegung gebunden ist. So erstreckt sich das Gebiet des Bewegungssehens bis etwa 100 Reizwechsel pro sec., was einem "Moment" von $1/50$ sec. entspricht. Totale Verschmelzung erfolgt oberhalb 120, also $1/60$ sec. Die untere Grenze für Bewegungssehen ist nicht festgestellt worden, jedenfalls ergaben 5 Reizwechsel pro sec. noch Reaktionen auf Bewegung (Fig. 3). Wenn zwei Sektoren, ein schwarzer und ein weisser innerhalb $1/50$ sec. dieselbe Retinastelle des Kampffisch-eyes reizen, so werden sie nicht mehr getrennt perzipiert, es wird also keinerlei Bewegung wahrgenommen, das zeitliche Auflösen hört auf.

(c) Das zeitliche Auflösen und die Latenzzeit

Das räumliche Auflösen ist die Leistung, zwei gleichzeitig gegebene Punkte im Raum getrennt wahrzunehmen (Sehschärfe). Sie ist für den Grad der Empfindlichkeit für Ortsgliederung massgebend. Ist dieses Vermögen gering, so werden nur grobe Gliederungen erkannt, die feineren Unterschiede verschmelzen zu homogenem Eindruck. Für die Formwahrnehmung ist neben dem räumlichen Auflösen auch die Farbunterschiedsempfindlichkeit (Qualitätsauflösungsvermögen) verantwortlich, die bei gleicher Gliederung umso leistungsfähiger ist, je geringer sich ein Glied vom Hintergrund abzuheben braucht um noch wahrgenommen zu werden. Ganz ähnlich ist der Begriff des zeitlichen Auflösens zu verstehen. Je kürzer die Dauer in der zwei Reize getrennt wahrgenommen werden, umso feiner ist diese Auflösung, umso feinere zeitliche Gliederung kann wahrgenommen werden, was in erster Reihe der Wahrnehmung von Bewegungen und Veränderungen in der Umgebung zu gute kommt. Der Begriff "Moment" lässt sich auf den der Refraktärphase (s. oben) und den der Chronaxie zurückführen. Das mit dem Begriff "Moment" gemeinte ist in Differenzen im zeitlichen Auflösungsvermögen ausdrückbar. Da das zeitliche Auflösen besagt, wie schnell zwei Reize aufeinander folgen können um getrennt wahrgenommen zu werden, hängt es mit der vielerörterten Frage der Latenzzeit der Erregung zusammen, das heisst der Zeit, die vom Eintreffen des Reizes im Rezeptor bis zur Wahrnehmung desselben verläuft. Weil die Methoden zur Untersuchung dieser Latenzzeit Phänomene anwenden, die in das Gebiet der Bewegungswahrnehmung gehören, soll hier kurz über sie berichtet werden.

Tritt in einem Ausschnitt ein bewegter Leuchtpunkt auf, so erscheint er nicht am Ort des Auftretens, sondern in die Bewegungsrichtung verschoben. Ist der Leuchtpunkt schwach, so ist diese Verschiebung grösser als bei intensivem Licht. Diese Erscheinung weist auf Unterschiede in der Latenzzeit hin. Es ist natürlich nicht möglich aus dieser Erscheinung auf den absoluten Betrag der Empfindungszeit zu schliessen, wie das in der Schule von Fröhlich getan wird (Fröhlich, Monjé, 1929), sondern nur auf Zeitunterschiede. Denn soll man den Leuchtpunkt deshalb in die Bewegungsrichtung verschoben sehen, weil der Punkt, bis er eindrucksmässig erscheint, inzwischen physikalisch bis an die Stelle gelangt, wo er gesehen wird, so ist es unverständlich wieso der Punkt überhaupt erblickt werden kann. Offenbar bedarf der Punkt auch an jener Stelle der Latenzzeit, wo er gesehen wird und inzwischen wandert er doch wieder weiter. Oder man müsste annehmen, dass nur der am Rand auftauchende Punkt der Latenzzeit bedarf, der an der gesehenen Stelle befindliche dagegen keine Latenzzeit hat (Metzger, 1932). Rubin (1929) zeigt, dass die Verschiebung von der Grösse der ganzen Bewegungsbahn abhängt, also auch von derjenigen Stelle, wo der Punkt später verschwindet. Solcher antizipatorischen Wirkungen werden wir noch mehreren begegnen. Obwohl man nicht sieht wann der Punkt verschwinden wird, ist die Länge der Bahn für das Ausmass der Verschiebung der phänomenalen Auftauchstelle vom physikalischen Rand ausschlaggebend. Bei ganz schmalen Ausschnitten ist die Verschiebung gleich Null.

(Vergleiche auch IV.) Dasselbe gilt für das Verfahren von Hazelhoff u. Wiersma, 1925.

Das Fröhlich'sche Phänomen beruht nun darauf, dass die Latenzzeit eines plötzlichen Reizes länger ist als die eines kontinuierlichen. Der plötzliche Reiz findet keinen Erregungszustand im Sinnesektor (Nervensubstanz vom Aufnahmeapparat bis zum kortikalen Zentrum) vor, und die zugehörige Erregung muss sich auf einem sozusagen neutralem Feld ausbilden. Demgegenüber lässt ein kontinuierlicher Reiz die zugehörige Erregung in jeder Phase aus dem im Sinnesektor vorhandenen Vorgang sich entwickeln.

Eine ähnliche antizipatorische Wirkung wie beim Fröhlich'schen Phänomen ist in einer anderen Beobachtung von Rubin (1932) enthalten, auf die wir ebenfalls im

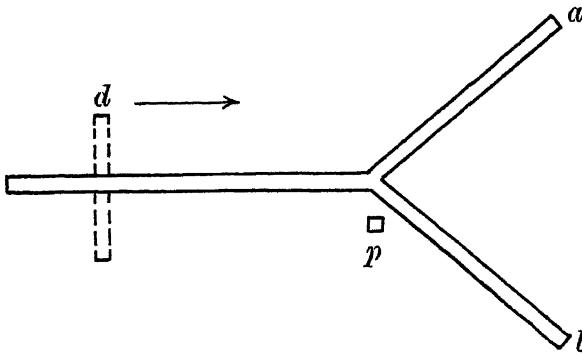


Fig. 4. Schema der Anordnung des Abrundungsphänomens von Rubin. Der Lichtstreifen *d* wandert in Richtung des Pfeiles. Der Ast *a* oder *b* ist verdeckt. *p* zeigt den Augenblick des Richtungswechsels an, mit dem der Anfang der Abrundung zu vergleichen ist.

Abschnitt IV zurückkommen. Betrachtet man einen leuchtenden Punkt in gleichmässiger Bewegung auf einer Bahn zweier Geraden im Winkel von 150° , so sieht man keine geknickte Bahn, sondern einen rundlichen Bogen. Die Rundung der Bahn erfolgt schon vor dem Knick obwohl dieser nicht zu sehen ist. Besteht die Bahn aus einem horizontalen und einem aufwärts gerichteten Schenkel, so biegt sich die Bahn vor dem Knickpunkt nach oben, ist der Schenkel nach unten gerichtet, so biegt sie sich nach unten, noch bevor der Leuchtpunkt den Knick phänomenal erreicht, wie das einfach zu beweisen ist, wenn man unter der Knickstelle einen unbewegten Leuchtpunkt anbringt (Fig. 7). Die Lage des bewegten Punktes vor dem Knick ist also von den später einzunehmenden Lagen abhängig. Offenbar ist gleichzeitig mit dem Wahrnehmen der Biegrichtung der Punkt physikalisch schon über den Knick hinausgelangt, der ausgelöste Erregungsvorgang aber noch nicht so weit zentralwärts geleitet, dass der Punkt an der Stelle zu sehen wäre. Es müssen also Erregungsvorgänge, denen Erlebnisse entsprechen mit solchen jüngeren, die in der Peripherie erst eben ansetzen irgendwie in Wechselbeziehung geraten. Vergleiche die Hypothese der tonischen Vormeldung in Abschnitt IV.

III. DIE SCHEINBEWEGUNGEN

Die bis jetzt erwähnten Versuche haben gemeinsam, dass für das Bewegungssehen das Verschieben eines abgegrenzten Lichtbereichs auf der Retina verantwortlich gemacht werden kann. Das ist aber nicht das physiologische Korrelat schlechthin. Wir haben schon Fälle kennen gelernt wo trotz vorhandener Lichtverschiebung keine Bewegung auftrat, so z. B. bei sehr raschem Wechsel der Reize, der zur Verschmelzung führt oder beim Fröhlich'schen Phänomen, wo die Bewegung über einen Teil der Bahn nicht gesehen wird. Auch in der sogenannten induzierten Bewegung (vgl. III, (1) (e)) erscheint das sich im Verhältnis zum Auge verschiebende Feld in Ruhe, wobei ein im Verhältnis zum Auge unbewegtes Objekt bewegt erscheint und dementsprechend behandelt wird (was biologisch meist zum Erfolg führt, weil das Objekt auf das Feld bezogen tatsächlich seine Stelle ändert). Wird das ganze Sehfeld verschoben, so kommt es oft zum Eindruck als ob der Wahrnehmende sich im ruhenden Feld bewegte. Diesen Fällen sind diejenigen entgegenzustellen, in welchen eine Bewegungswahrnehmung vorhanden ist, obgleich keine Lichtverschiebung auf der Retina auftritt. Ausser dem Bewegungsnachbild, das nach längerem Einwirken einer Bewegung in einer Fortdauer dieser Bewegung in entgegengesetzter Richtung besteht, gehört die Kinematoskopie zu diesen Fällen. Sukzessiv auftretende diskrete Lichtreize erzeugen unter geeigneten Zeitbedingungen einen Bewegungseindruck, den sogenannten kinematoskopischen Effekt, der von einer realen Bewegung die ihrerseits mit Lichtverschiebung auf der Retina einhergeht, nicht zu unterscheiden ist, weder im Erleben des Menschen, noch im Verhalten mancher Tiere. Das in der Kinotechnik übliche Verfahren verwendet ausser dem kinematoskopischen Effekt auch das Phänomen der Verschmelzung um eine Verdeckung der Bilder während ihres tatsächlichen Weiterrückens unbemerkt vornehmen zu können.

Da sich diese sogenannten Scheinbewegungen am Menschen genauer studieren lassen, so scheint es mir geboten, in diesem Abschnitt die Reihe umzukehren und die am Menschen und an Wirbeltieren gewonnenen Ergebnisse vor den an Avertebraten ausgeführten Untersuchungen zu behandeln.

(1) DER MENSCH UND DIE VERTEBRATEN

(a) *Kinematoskopie beim Menschen*

Der Mensch sieht die aufeinanderfolgenden ruhenden Bilder eines Filmstreifens einheitlich bewegt. Man untersucht diesen kinematoskopischen Effekt indem man eine möglichst einfache Reizkonstellation wählt, z. B. das Aufeinanderfolgen zweier Lichtpunkte. Durch mannigfache Variation der Zeitfolge und anderer Faktoren hat man verschiedene Gesetzmässigkeiten feststellen können. So erkannte Bourdon und später genauer Wertheimer (1912), dass bei sehr rascher Abfolge keine Bewegung sondern eine Art Verschmelzung auftritt, das sogenannte Simultanstadium, so benannt weil beide Endreize gleichzeitig zu sehen waren. Ist die Abfolge langsam und ist verhältnismässig viel Pause eingeschaltet zwischen die beiden

Reize, so ergibt sich das Sukzessivstadium, wobei beide Punkte getrennt nacheinander erscheinen, die Auflösung eine vollkommene ist. Auch im Sukzessivstadium ist ein Übergang zwischen beiden Reizen phänomenal gegeben. Sind beide Reize verschieden, so erlebt man das Hellerwerden, die Formwandlung u.s.w., ohne dass man in der Übergangszeit etwas anderes als gerade dieses Verhältnis der beiden Reize erlebte. Diese Übergangserlebnisse (Schumann, 1904) ermöglichen den sinnlichen Sukzessivvergleich. Gibt man zwei zu vergleichende Objekte in kinematoskopisch optimaler Folge so ist der Übergang ganz anschaulich, man sieht einen einzigen sich verwandelnden Reiz. Betrachtet man die beiden Glieder der Müller-Lyer'schen Figur nacheinander (Fig. 5) so erlebt man bei entsprechender Reihenfolge ein Grösserwerden. In kinematoskopischer Folge sieht man das Wachsen des Mittelstriches trotzdem er objektiv unverändert bleibt. Diese von

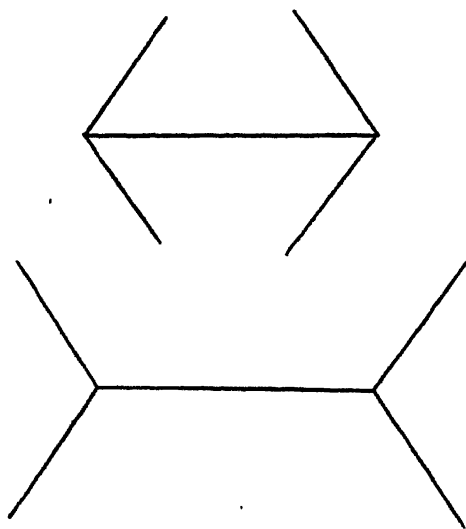


Fig. 5. Die Müller-Lyer-Figur, in der die objektiv gleichlangen vertikalen Streifen ungleich erscheinen. In kinematoskopischer Exposition entsteht der Eindruck des Wachsens.

Benussi (1912) beobachtete Erscheinung wurde von Korte (1915) α -Bewegung genannt. Nach Wittmann (1921) besteht diese Bewegung auch bei kontinuierlicher Verschiebung einer sog. Jastrow-Figur (Kreisringsegment). Dem Sukzessiv- und dem Simultanstadium gegenüber bezeichnet man Zeitbedingungen unter denen einheitliche Bewegung wahrgenommen wird, als Optimalstadium. Viel Missverständnis stammt auf diesem Gebiet daher, dass die Forscher das Optimalstadium nicht genau festlegten. Aus diesem Grunde haben manche Forscher gemeint, dass die Bewegung nur vorgestellt werde und eigentlich garnicht sichtbar wäre. Solche Beobachtungen beruhen aber allein auf technisch unvollkommener Erzeugung des kinematoskopischen Effekts. Nach Waals u. Roelofs (1930) ist das unmittelbare Aufeinanderfolgen zweier sekundenlanger Lichter als optimale Zeitfolge anzusehen. Ich habe diesen Befund etwas modifiziert verallgemeinert, indem ich feststellte, dass lange Expositionszeiten und sehr viel kürzere Unterbrechungsdauer—etwa 10—

250—für das Erzeugen des Optimalstadiums nötig sind (Schiller, 1933). Dabei werden Effekte erzielt, die bei gleichzeitigem Darbieten reeller Bewegung von letzterer nicht unterschieden werden. Die Richtigkeit dieser Feststellung wird weitgehend begründet in den neueren Versuchen der beiden Holländer (Waals u. Roelofs, 1933). Sie bauen eine Anordnung die ermöglicht (1) zwei räumlich getrennte Reize beliebig lang zu exponieren, (2) einen Reiz in reeller Verschiebung zwischen den beiden Endreizen darzubieten. Wird nur (2) eingeschaltet, so entsteht eine ungünstige Bewegungswahrnehmung (die Bewegung erscheint verschwommen); werden aber die ruhenden Endphasen mit Hilfe von Anordnung (1) dazugefügt, so tritt gute Bewegung auf. Bleibt (1) weg so muss die Bewegung von (2) langsamer oder auf einer grösseren Bahn erfolgen um deutlich zu werden. Auch der momentane Wechsel der beiden Ruhepunkte von (1) führt zu optimaler Bewegung. So ist also auch für die reelle Bewegung die ruhende Endphase von Wichtigkeit, was besagt, dass unter Umständen die Wahrnehmung einer realen Bewegung ein reines kinematoskopisches Sehen ist, das durch die Verschiebung des Lichtes auf der Netzhaut nicht im geringsten begünstigt wird. Hier soll erwähnt werden, dass der Effekt auch dann auftritt, wenn die beiden Endreize getrennten Augen zugeführt werden: haploskopischer Versuch von Benussi (1925), Langfeld (1927), Piéron (1934), vereinfachende und verdoppelnde Kinematoskopie von Pikler (1917).

Linke dachte, dass der Bewegungseindruck "psychisch" entsteht, d. h. Vorstellungen ergänzen das physiologisch Gegebene im Sinne der Erfahrungen über Identität des Bewegten. Die Identität zweier diskreter Lichter wird durch die Produktion einer Bewegungsverbinding auf dem Vorstellungswege gesichert. Zum Beweis dieser Anschauung, die einer Leugnung des physiologischen Korrelats des kinematoskopischen Sehens gleichkommt, wird meistens zu den alternativen kinematoskopischen Konstellationen zurückgegriffen (Linke, 1907; Benussi, 1918; Wittmann, 1921; de Silva, 1929; Neuhaus, 1930; Squires, 1931). Exponiert man ein Kreuz mit einem vertikalen und einem horizontalen Balken und rasch nach dessen Erlöschen ein gleiches Kreuz mit zwei diagonalen Balken konzentrisch, so besteht bei einer Neigung von 45° der beiden Kreuze zueinander gar keine Gesetzmässigkeit, sondern der eine Beobachter sieht eine Bewegung im Sinne des Uhrzeigers, der andere in entgegengesetztem Sinne, und es gibt Beobachter, die abwechselnd die eine oder die andere Bewegung sehen. Durch systematische Variation von Bewegungsalternativen habe ich nachzuweisen versucht, dass die Art der Bewegung in solchen "Täuschungsfällen" nicht beliebig ist, sondern bei Variation objektiver Bedingungen sich voraussagen lässt (Schiller, 1933). Färbt man z. B. die Balken in der Rechtsdrehung immer dunkler, so erfolgt die Bewegung immer nach rechts mit Beibehaltung der Farben. Durch diese Alternativversuche ist eine Reihe von Gesetzmässigkeiten miteinander in Zusammenhang gebracht worden, wie folgende Ausführungen zeigen.

Die Tendenz, die den kinematoskopischen Effekt determiniert, wurde von mir Tendenz zur totalen Angleichung genannt (siehe auch Koffka, 1935). Die erste Figur einer kinematoskopischen Konstellation gleicht sich der zweiten in jeder Beziehung an: weichen beide Figuren nur in der Raumstelle voneinander ab, so

entsteht Bewegung, wenn sie aber auch der Qualität nach verschieden sind, so tritt mit Verwandlung gepaarte Bewegung auf. Die zweite Figur entsteht in allen ihren Momenten anschaulich aus der ersten. Diese Tendenz ist zuerst von Pikler ausdrücklich formuliert worden.

Die Art der totalen Angleichung ist von weiteren Tendenzen abhängig. Ist die totale Angleichung auf zwei Bewegungsbahnen möglich, so erfolgt sie auf der kürzeren (Wertheimer, 1912) oder allgemeiner auf der einfacheren (Schiller, 1933). Je einheitlicher, symmetrischer, "besser gestaltet" die eine Bewegungsbahnalternative ist, umso eher wird sie bevorzugt.

Neben der Tendenz zur einfachsten Bahn ist die Tendenz zur Beibehaltung der zeitersten (an erster Stelle exponierten) Gestalt ausschlaggebend. Figurale Verzerrungen werden nach Möglichkeit vermieden, selbst wenn die Angleichung ohne Verzerrung nur auf dem komplizierteren von zwei Bahnen möglich ist. Beibehaltungstendenzen auf der günstigen Bahn sind auch von Ternus (1926) festgestellt worden.

Sind mehrere Teilmglieder in der Gestalt vorhanden, so erfolgt die Bewegung in die Richtung der gleichgefärbten, gleichgeformten, gleichgrossen Figurenglieder. Gleiche Teilmglieder bilden in der simultanen Figuralgliederung Gruppen (Wertheimer), im sukzessiven ziehen sie sich gleichsam an. Eine Anziehungs- bzw. Verschmelzungstendenz gleicher und ähnlicher Glieder einer Reihe ist zuerst von Ranschburg (1902—genauer 1913) ausgesprochen worden. Dabei ist merkwürdig, dass die Bewegung in der Richtung der gleichen Glieder erfolgt, noch bevor diese am Ende der Bahn erscheinen (Fig. 6). Hier haben wir also wieder mit einem Fall von antizipatorischer Wirkung in der Bewegungswahrnehmung zu tun. Diesen Eigenschaften des kinematoskopischen Effekts wird eine Bedeutung für die physiologische Theorie des Bewegungssehens, Abschnitt IV, zukommen.

Die Tendenz zur einfacheren Bahn ist nicht nur für kinematoskopische sondern auch für reelle Bewegung festgestellt worden. Rubin (1927) lässt auf einem in horizontaler Richtung sich bewegenden Rad nur einen excentrischen Leuchtpunkt sehen. Man sieht eine Bewegung auf zyklöider Bahn. Wird ein zweiter Punkt exponiert so merkt man zwei sich kreuzende zyklöidale Bahnen. Werden aber drei oder mehr Lichter gezeigt, so ergibt sich—wenn sie nicht eng aneinander gruppiert sind—ein ganz neues Phänomen. Man sieht eine Dreieckfigur die sich rotierend fortbewegt. Die Wahrnehmung ist nicht imstande so viele unabhängige komplizierte Bahnen auseinanderzuhalten und behandelt das Dreieck sozusagen wie einen Kreis. Metzger (1934 a) hat ähnliches in Alternativversuchen gezeigt. Kreuzen sich zwei bewegte Punkte an einer Stelle, so ist bei qualitativer Gleichheit der Punkte nicht möglich zu definieren, welcher Punkt an welcher Bahn weitergeht. Trotzdem erscheint wahrnehmungsmässig unzweideutig, welcher Punkt in welcher Richtung weitergeht; die Punkte scheinen immer in der Richtung der bestgestalteten Fortsetzung ihrer eigenen Bahn zu gehen (Fig. 7). Wird der Schatten einer sich umdrehenden Drahtfigur betrachtet, so sieht man Flächenbewegung, wenn die Bahn einfach genug ist, aber Tiefenbewegung, wenn die Flächenbewegung zu kompliziert wäre oder wenn diese allzu grosse Verzerrungen der gedrehten Gestalt

erforderte (Metzger, 1934 b). Die nämlichen Tendenzen habe ich in den kinematoskopischen Alternativversuchen festgestellt (Schiller, 1933). Ist in einer kinematoskopischen Konstellation eine Bewegungsbahn sowohl in der Fläche wie in der Tiefe möglich, so erfolgt die Tiefenbewegung, wenn diese Bahn die geo-

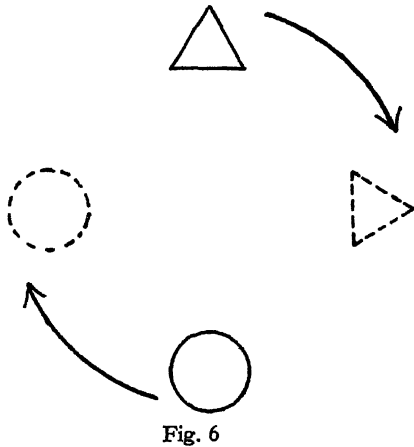


Fig. 6

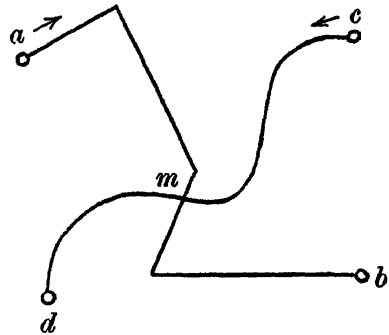


Fig. 7

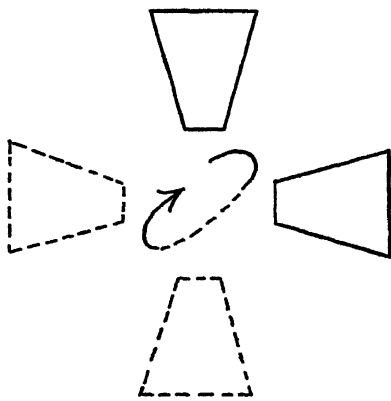


Fig. 8

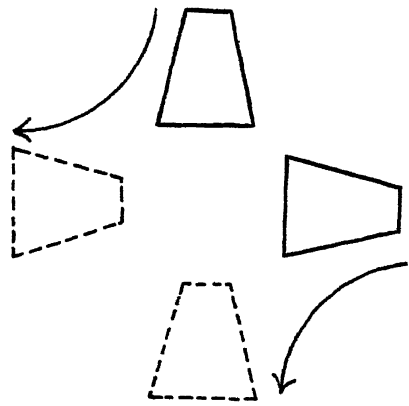


Fig. 9

Fig. 6. Alternative kinematoskopische Konstellation, die zu einer Bewegung im Sinne der Figurengleichheit führt. Ausgezogene Linie zeigt erste, punktierte Linie die zweite Exposition. Die Pfeile deuten die gesehene Bewegungsrichtung an (geometrisch ist auch die umgekehrte Richtung möglich). (Nach Schiller.)

Fig. 7. Die Bewegungsbahnen von *a* und von *c* schneiden sich in *m*. Treffen sich die Punkte in *m* wobei sie zur Deckung kommen, so sieht man *a* in Richtung von *b*, *c* in Richtung von *d* weiterwandern, obwohl die entgegengesetzte Fassung theoretisch möglich ist. (Nach Metzger.)

Fig. 8. Alternative Konstellation, die kinematoskopisch exponiert (vgl. Legende zur Fig. 6) zur Tiefenbewegung führt, wie der Pfeil andeutet. (Nach Schiller.)

Fig. 9. Alternative Konstellation, bei der Flächenbewegung bevorzugt ist. (Nach Schiller.)

metrisch einfachere ist oder wenn dadurch die Gestalt vor Verwandlungen und Verzerrungen bewahrt bleibt (vgl. Fig. 8). Eine Zerreißung der aus selbstständigen Gliedern gebauten Gestalt erfolgt nur wenn die Bewegung bei Beibehaltung dieser Gestalt auch in der Tiefe viel zu kompliziert ist, wie etwa in der Fig. 9. Nach

Calavrezo (1934) wird das übermässige Wachsen in einer kinematoskopischen Konstellation durch perspektivische Tiefenbewegung ersetzt. So erscheint also der Stereoeffekt von der Art der Bewegungsgestalt abhängig zu sein, in dem Sinne, dass er auftritt, wenn die einfachste dynamische Gliederung durch ihn gesichert ist. Ähnlich hat Koffka (1930) für den räumlichen Eindruck von Flächenzeichnungen die einfachste geometrische Gliederungsart, d. h. die Tendenz dazu, verantwortlich gemacht. Zur Verifizierung dieser Hypothesen könnten Versuche an Tieren herangezogen werden, nach dem Muster wie Herter (1930) das Formunterscheidungs- und körperliche Sehvermögen der Fische nachgewiesen hat.

Nach den Ergebnissen der Alternativversuche steht soviel fest, dass das kinematoskopische Bewegungssehen kein Produkt des Vorstellens ist, das beliebig verlaufen könnte. Es gibt nur ganz spezielle Fälle von sog. Kippfiguren (wie im Simultanfeld das bekannte maltesische Kreuz) bei denen die Bedingungen sich sozusagen die Wage halten. Die Angleichung einer figuralen Konstellation an die andere erfolgt auf Grund der grösstmöglichen Stetigkeit und Einheitlichkeit, eines Gesetzes, das nach gestalttheoretischer Forschung für die zeitliche Gliederung der Wahrnehmung überhaupt bestimmend zu sein scheint. Obwohl schon Mach (1875) und Exner (1891) nach dem physiologischen Korrelat des Bewegungssehens suchten, glauben manche moderne Autoren (Dimmick, 1920; Higginson, 1926 a; de Silva, 1929) die Existenz eines solchen Korrelats leugnen zu müssen (vgl. IV).

(b) *Kinematoskopie bei Phoxinus*

Gegen die Produktionstheorie des Bewegungssehens sprechen auch die neuesten Feststellungen, dass niedere Vertebraten die kein corticales Sehzentrum besitzen, zu kinematoskopischer Bewegungswahrnehmung fähig sind. Das Vorhandensein des kinematoskopischen Sehens an Fischen wurde in zwei gleichzeitigen Arbeiten mit Hilfe ganz verschiedener Methoden ermittelt.

Gaffron (1934) wendete ihre Drehzylinderanordnung an. Wie in II, (1) gesagt, gingen die Fische mit der Bewegung mit, wenn die Bewegung schnell genug und auf ein grosses Feld ausgedehnt erschien. Wurde diese Bewegung mittels intermittierender Beleuchtung in eine stroboskopische verwandelt (viele schwarz-weiße Streifen sukzessiv in verschiedenen Lagen gezeigt), so gingen die Fische auch mit, und zwar je nach der Unterbrechungsdauer in der einen oder anderen Richtung, wie der Mensch die Richtung gesehen hat. Man sieht die Bewegung in derjenigen Richtung, wo die benachbarten Balken die kleineren Sprünge machen. (Vergleiche das über die Bahnalternativen gesagte.) Gaffron erwähnt auch, dass Hunde sich im Kino lebhaft interessiert zeigen.

In meinen Versuchen (Schiller, 1934) bin ich nicht von der komplizierten stroboskopischen, sondern von der einfachen kinematoskopischen Konstellation ausgegangen und arbeitete mit der Dressurmethode an *Phoxinus*. Ausgehend von den drei Stadien des kinematoskopischen Effektes variierte ich die Zeitbedingungen so, dass bei gleich häufigem Wechsel und gleich langer Bahn Optimal-, Simultan-, und Sukzessivstadium gegeneinander auszuspielen waren (vgl. Fig. 10). Die Unterscheidung ist in allen Fällen rasch gegangen und optimale liess sich durch reelle

Bewegung ebenso ersetzen wie umgekehrt. Eine Unterscheidung von optimal und reell aber gelang auch in zwei bis dreifacher Zeit nicht, gleichwohl, ob diese Dressur den anderen voranging oder folgte.

So ist also gesichert erwiesen, dass auch für niedere Wirbeltiere das diskrete Auftauchen von ruhenden Lichtreizen unter Umständen ausreichende Bedingung des Bewegungssehens darstellt. Ferner steht fest, dass kinematoskopisches Bewegungssehen nicht an das Vorhandensein eines entwickelten Kortex gebunden ist.

Einige Beobachtungen aus der Pathologie der menschlichen Wahrnehmung sind manchmal so interpretiert worden, als ob der kinematoskopische Effekt an intaktes Funktionieren der höchsten Zentra gebunden wäre. Bei einem von Gelb u. Goldstein (1920) untersuchten Hirngeschädigten war trotz vorhandenen Bewegungssehens kein kinematoskopisches Sehen vorhanden. Ein paralleler Fall ist von Stein u. Beringer (vgl. Stein u. Mayer-Gross, 1928) angegeben, die feststellten, dass obzwar im Meskalinrausch auch ruhende Gegenstände bewegt erscheinen, die stroboskopische Konstellation dennoch zur Wahrnehmung gleichzeitiger diskreter Reize führt. Zur richtigen Interpretation dieser Beobachtungen muss man bedenken, dass durch die betreffende Vergiftung bzw. Erkrankung der Nervenpartieen, vermutlich der Chronaxiewert und das Refraktärstadium derselben gestiegen ist, so dass der Kranke Gleichzeitigkeit erlebt, wo der Gesunde Bewegungszusammenhang wahrnimmt. Es ist aber natürlich möglich, dass beim Menschen eine Funktion an höhere Zentra gebunden ist, die beim Fisch von niederen Organen besorgt wird (progressive Zerebration).

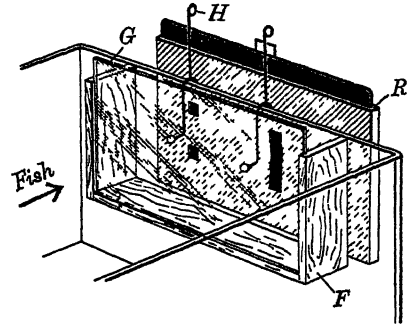


Fig. 10. Anordnung für *Phoxinus laevis* zur Unterscheidung von Bewegungsphänomenen. F Futterkasten; G, Glaswand; H, Futterhaken; R, Reizkarte. (Nach Schiller.)

(c) Induzierte Bewegung

Eine zweite Klasse von Scheinbewegungen ist bekannt unter dem Namen "induzierte (relative) Bewegung". Sie wird am besten verständlich an einem kinematoskopischen Versuch von Ehrenstein (1925). Ist ein Punkt beidemal auf derselben Raumstelle (bei Fixierung durch das Auge), aber das erstemal auf dem einen Rande, das zweitemal auf dem anderen Rande eines verlagerten Quadrats kinematoskopisch exponiert, so nimmt man keine Bewegung des Quadrats, sondern eine solche des Punktes wahr. Entsprechendes gilt auch bei kontinuierlicher Verschiebung. Bewegen sich zwei Objekte gegeneinander, d. h. ändert sich der Abstand zwischen beiden, so ist der Bewegungseindruck nicht davon abhängig, ob die, bzw. welches der Objekte im Verhältnis zum Blickpunkt sich bewegen bzw. sich bewegt, sondern lediglich von der figuralen Gliederung. Ruht das eine Objekt und befinden sich beide auf einem ruhenden Grund, so erscheinen entweder beide bewegt, oder aber nur das eine. Erscheint nur ein Objekt bewegt so ist es dasjenige,

das als Figur sich vom Grunde abhebt, wie der Mond am wolkigen Himmel. Sind beide figurartig und sind sonst keine Gegenstände vorhanden, so bewegt sich die fixierte Figur, also diejenige, die sich im Verhältnis zum Auge nicht bewegt (Thelin, 1927; Duncker, 1929).

Wird auf einem leeren leuchtenden Feld plötzlich eine Figur exponiert, so erscheint diese von einem Mittelpunkt ausstrahlend in plötzlicher Ausdehnung. Diese sog. Gamma-Bewegung zeigt den Übergang vom unprägnanten Fleck zur gegebenen Figur (Korte, 1915; Engel, 1928; Harrower, 1929). Auch die Gamma-Bewegung bleibt unter Umständen unbemerkt und induziert Bewegung auf eine auf dem Feld befindliche kleine Marke (Newman, 1934). Ist der Ausmass der Bewegung im Verhältnis zum Auge oder Körper bedeutsam, so sind die Gesetze die von Duncker (1929) festgestellt sind auch für den Organismus als Objekt gültig. Ist der Beobachter selbst eine ruhende Figur im umschliessenden bewegten Feld, so empfindet er sich selbst als bewegt im ruhenden Feld. Das erlebt man bekanntlich wenn man von der Brücke das treibende Eis in dem Fluss betrachtet. Beugt man sich nach vorne, so dass das ganze Gesichtsfeld von Bewegung erfüllt ist, so erfolgt gleich vestibuläres Schwindelgefühl. Das Streifenmuster ist für die Untersuchung der Bewegungswahrnehmung als Nachahmung dieses Phänomens von Mach (1875 und früher) in die Sinnesphysiologie eingeführt worden.

In einer rotierenden Trommel mit Zaunmuster empfindet man Gedrehtwerden wenn die Bewegung schnell genug ist. Dieses Erlebnis tritt eher ein wenn man einen ruhenden Gegenstand vor dem Zaun fixiert. Bei langsamer Rotation ist Bewegungssehen und Gefühl des Gedrehtwerdens abwechselnd gegeben, wobei erstes mit den langsamen, zweites mit den raschen Phasen des optokinetischen Nystagmus (Korrekturbewegungen mit den Augen) zusammenfallen soll (Fischer u. Kornmüller, 1930). Die motorischen Reaktionen fallen bei Verschmelzung der Streifen aus und dabei entsteht der Eindruck des kontinuierlichen Gedrehtwerdens (Vogel, 1931). Nach v. d. Waals (1933) sind Schwindelreflexe auch bei stroboskopischer Exposition vorhanden.

Vertebraten, vor allem Fische und Eidechsen, scheinen im Drehzylinder Bewegung der Umgebung wahrzunehmen (vgl. II, (1)). Ist die Bewegung zu schnell, so tritt (bei Verschmelzung) gar keine spezifische Reaktion auf. Bei angemessener Bewegung sind die Reaktionen nicht als Lagekorrekturen zu verstehen, da sie bei einmaligem, plötzlichem Verrücken bei Fischen ausbleiben (Gaffron, 1934). Bei Eidechsen hat Schlieper (1927) nystagmusartige Augen- und Kopfbewegungen festgestellt, ebenso bei einigen Dekapoden die mit ihrem Augenstil nystagmusartige Bewegung ausführten (vgl. II, (1) und III, (2)).

Auch in dem Fall von Blickverschiebung im ruhenden Raum ist für den Menschen keine der Lichtverschiebung zugeordnete Bewegungswahrnehmung vorhanden, sondern es ist bei Empfindung der eigenen Augenbewegung der Eindruck der Ruhe in der Umgebung gegeben. Sitzt man in einem Zimmer das mit verschiedenen Gegenständen erfüllt ist und wandert man mit den Augen von der einen zur anderen Ecke, so verschieben sich die Bilder auf der Netzhaut. Man merkt, dass das Auge im unbewegten Raum umherschweift. Bei Augenbewegungen

tritt Bewegungssehen nur dann auf wenn die Myosensationen ausgeschaltet sind, wie bei den passiven Augenbewegungen des Menschen (Schilder, 1912) (vgl. auch II, (1)) oder wenn mit der Augenbewegung relative Lichtverschiebungen der Sehdinge einhergehen, etwa wenn ein naher und ein ferner Gegenstand sich relativ verschieben. Solche relativen Verschiebungen erzeugen den Eindruck zweier entgegengesetzter Bewegungen in der Landschaft während der Eisenbahnfahrt (Poschoga, 1927).

Der Bereich des scharfen Sehens hebt sich als Figur vom unscharf gesehenen erfüllten Sehfeld ab. Bei Blickbewegung verschieben sich die unscharfen Gegenstände um den Bereich des scharfen Sehens. Ist der Blick in bezug auf die Objekte lokalisiert, so werden die Objekte unbewegt gesehen, der Bereich des scharfen Sehens dagegen an den Objekten entlang bewegt und wir merken also nicht die Objekte z. B. nach links gerutscht, sondern den Blick nach rechts verschoben (Duncker, 1929). Für die Lokalisation der Objekte in bezug auf den Körper ist es wichtig zu wissen ob der Blick z. B. gerade oder seitlich gerichtet ist. So dient also die Bewegungsinvarianz bei Blickverschiebung zur entsprechenden Orientierung der Organismen in ihrem Lebensraum.

(2) DIE AVERTEBRATEN

(a) *Induzierte Bewegung bei Aeschna*

Wie wir sahen ist es neuerdings einigen Forschern gelungen die behandelten Bewegungstäuschungen auch für einige Tiere unter den Vertebraten gültig zu erweisen, womit eine grundlegende Tat für die allgemeine Perzeptionslehre getan worden ist. Ebenso wie das Formensehen auch in ihren sog. Täuschungen die gleiche Gesetzmässigkeit zeigt wie beim Menschen, nach Untersuchungen von Révész (1924) und anderen an Hühnern, von Herter (1930) an Fischen, erscheint auch das Bewegungssehen in vieler Beziehung prinzipiell in verschiedensten Tiergattungen nach der gleichen physiologischen Struktur vor sich zu gehen, umsomehr als einige Scheinbewegungen auch für Tiere mit einem prinzipiell vom Menschenauge verschiedenen Lichtsinnesorgan, dem Komplexauge, nachgewiesen worden sind.

Gaffron (1934) ist es gelungen den Nachweis induzierter Objektbewegung bei der Larve *Aeschna* zu bringen. *Aeschna* schnappt nach sich abhebenden, bewegten Körpern und nur nach solchen. Reaktionen auf ruhende Körper kommen ganz selten vor, so befindet sich die Larve dabei immer in Bewegung (Baldus, 1927), und zwar in passiver, z. B. beim Herabsinken, die also nicht mit Muskelsensationen einhergehen. Kommt der *Aeschna*, die sich im Drehzylinder befindet, ein ruhender Fleck in die Fixierichtung, so macht sie in der optomotorischen Bewegung plötzlich halt, fixiert und schnappt nach dem Fleck. Der Fleck muss sich also im Augenblick des Zuschnappens für das Tier in Bewegung befunden haben. Der Versuch lässt sich regelmässig wiederholen, so dass eine Erklärung durch die gelegentlich vorkommenden Schnappreaktionen auf ruhende Objekte ausgeschlossen ist. Klebt man einen Plastilinfleck auf die Wand des Aquariums mit *Aeschna*, so erfolgt kein

Schnappen, selbst wenn die Tiere den Fleck fixieren. Wird hinter dem Fleck ein Streifenpapier bewegt, so schnappen die Tiere, wenn der Fleck in Fixierichtung kommt nach dem objektiv ruhenden Fleck. Auch schnappen sie, wenn sie längere Zeit im Drehzylinder waren und das bewegte Muster verdeckt wird, noch einige zeitlang nach ruhenden Objekten, was eine Nachwirkung des Bewegungsreizes beweist, die nach Gaffron eine nahe Verwandtschaft mit dem menschlichen Bewegungsnachbild hat. Merkwürdigerweise dauert diese Nachwirkung viele Minuten und oft eine halbe Stunde, wogegen beim Menschen ein visuelles Bewegungsnachbild selten eine Minute erreicht. Da aus der *Aeschna*-Reaktion nichts über die Richtung der gesehenen Bewegung festzustellen ist, müssen die Vergleiche mit menschlichen Phänomenen vorläufig zurückhaltend sein. Der Fall scheint eher einen bedingten Reflex darzustellen.

(b) *Keine Kinematoskopie bei Arthropoden*

Gaffron versuchte den kinematoskopischen Effekt an Fliegen nachzuweisen. Leuchten in einem Zylinder benachbarte Balken der Reihe nach sukzessiv auf, so entsteht bei geeigneten Zeitbedingungen für den Menschen der Eindruck eines einzigen bewegten Lichtspaltes. In einem solchen Apparat ist es nicht gelungen bei Fliegen gerichtete optomotorische Reaktionen festzustellen. Gaffron wählte drei Anordnungen. Diejenige in der der Nachweis für Fische gelungen ist (vgl. II, (1)), führte zu widersprechenden Ergebnissen. In den beiden anderen Anordnungen gingen die Fliegen bei kontinuierlicher Bewegung mit, bei sukzessiver Reizung aber nicht. Dabei war in der einen Anordnung nur die Zeitlage von Belichtung und Pause variierbar, nicht der Abstand. In der anderen Anordnung war ein der Bewegungssehschärfe entsprechend berechneter Abstand vorhanden, doch war diesmal die Zeitlage von Belichtung und Pause zueinander nicht zu ändern. Aus diesen versuchstechnischen Gründen scheint mir das Ergebnis kein Nachweis für das Fehlen des stroboskopischen Sehens bei der Hausfliege zu erbringen, umsoweniger, als auch Fische in diesen beiden Anordnungen nach der Angabe von Gaffron nicht mitgingen.

Ich versuchte in anderweitig nicht veröffentlichten Versuchen den kinematoskopischen Effekt unter einfacheren Bedingungen an einem anderen Tier mit Facettenauge (*Galathea*) zu untersuchen, die bereits erwähnte Signalreaktion dieses Krebses anwendend (II, (1)).

Im Aquarium zu Rovigno standen mir 3 Exemplare *Galathea squamifera* zur Verfügung.¹ Die reagierten alle auf in der Luft über sie bewegte weisse und schwarze Gegenstände von etwa 4 cm.² (vgl. Fig. 1) und auch auf Lampenlicht das in einer Blende bewegt wurde, um das Sehen anderer bewegter Partien auszuschalten. Ist in einem Leuchtkasten nun ein entsprechend grosser Lichtfleck 1 sec. lang gegeben worden, und dann ein anderer 5 oder 10 cm. entfernter Fleck kinematoskopisch anschliessend (mit minimaler Pause, die praktisch 0 war) dann folgt das Tier dieser Bewegungsbahn nicht. Es zeigt sich höchstens eine Antennen-

¹ Für das freundliche Überlassen eines Arbeitszimmers danke ich den Herren Direktoren Prof. A. Steuer und Prof. M. Sella auch an dieser Stelle.

zuckung von 1–2 mm. im Augenblick des Aufleuchtens, des Wechselns und des Verlöschens. Dieselben Ergebnisse wurden erzielt mit 3 mal langsameren und 3 mal schnelleren Reizfolgen (die alle für den Menschen optimal erscheinen. Vgl. III, (1)). Selbst die Antennenzuckungen waren nur in den ersten 3–4 Versuchen festzustellen, nach dem Beginn oder nach irgendeiner herbeigeführten Störung, wo das Tier in Bereitstellung war, Antennen und Scheren leicht gehoben. In der gleichen Weise reagiert das Tier wenn man eine nicht-leuchtend in der Blende gehaltene Taschenlampe plötzlich aufleuchten lässt. In der kinematoskopischen Konstellation reagiert *Galathea* also nicht wie auf Bewegung, sondern wie auf aufleuchtende unbewegte Reize.

Auch bei teilweiser räumlicher Überdeckung der kinematoskopisch exponierten Lichtflecken war keine Bewegungsreaktion festzustellen. Sechs nebeneinanderstehende Lampen werden nacheinandergeschaltet mit Hilfe einer Kontakttrommel. (Die Anordnung wurde gleichsam als Übergang zur kontinuierlichen Bewegung so gewählt.) Es erfolgten in den ersten Versuchen jeder Versuchsstunde 4–6 Zuckungen der Antennen, wie beschrieben, und sonst nichts, gleich ob die Lichter mit scharfen Grenzen abgesetzt oder ungetrennt hinter einer Mattglas-scheibe mit Zerstreuungskreisen erscheinen.

Das eine Tier reagierte auf eine auf Draht befestigte Figur, deren Schatten auf ein an der Glaswand klebendes feuchtes Leintuch geworfen bewegt wurde. Wurde mit Hilfe von zwei Lampen der Schatten der Figur 2–12 cm. kinematoskopisch bewegt, so erfolgten auch hier in den ersten 3–4 Versuchen ungerichtete Antennenschläge, die nur im Augenblick des Lichtwechsels deutlich waren.

Die Reaktionen auf kontinuierliche Bewegung waren regelmässig, und folgten soweit anatomisch möglich auf der ganzen Bahn bei mässiger Geschwindigkeit, aber—entgegen Doffeins (1910) Befunde—ermüdeten auch diese Reaktionen bald. Jedenfalls ist es auch mit dieser Methode nahe gelegt, dass Tiere mit facettierten Augen kein kinematoskopisches Sehen besitzen.

Diese Befunde sind in keinem guten Einklang mit der klassischen Auffassung Exners (1891), wonach das Facettenauge in derselben vollkommenen Weise dem Bewegungssehen dient, wie das Linsenauge dem Formsehen. Denn wird das Mosaikauge tatsächlich, wie Exner jedenfalls für das Superpositionsauge (auch bei Dekapoden) annahm, durch die Irradiation des Lichtes über das betroffene Omma in benachbarte Sehelemente, in seiner Funktion sozusagen vereinheitlicht, so ist schwer zu verstehen, dass zeitlich unmittelbar aufeinanderfolgende, sich noch dazu räumlich teilweise überdeckende Reize, zu keinem einheitlichen Eindruck führen, sondern vielmehr allem Anschein nach diskret wahrgenommen werden. Jedenfalls reichen für das Linsenauge solche Bedingungen selbst bei scharfer Bildentwerfung aus, um einen Bewegungseindruck hervorzurufen. Der Unterschied in den beiden Leistungen scheint in zentralen Partien des Nervensystems zu liegen. Reichen noch die subkortikalen Zentren für eine Vereinheitlichung diskreter Erregungsvorgänge aus, so sind die Ganglien der Arthropoden scheinbar zu dieser Leistung nicht mehr fähig (vgl. IV). Dieses Ergebnis ist erstaunlich genug, da doch das Formsehen eine nicht geringe Vereinheitlichung diskreter Vorgänge (allerdings in simultaner

Gliederung) verlangt und diese vom Arthropodennervensystem bewerkstelligt wird (vgl. II, (1)). So scheint dieser negative Befund von Gaffron und von mir noch weiterer Nachprüfung bedürftig.

IV. ZUR PHYSIOLOGISCHEN THEORIE DES BEWEGUNGSSEHENS

Eine physiologische Theorie der Verhaltenstatsachen ist gegeben, wenn man ein physiologisches Korrelat findet, das die Zuordnung von Reizvariation zu Verhaltensvariation besorgen kann. Ist einer Reizverschiedenheit eine Verhaltensverschiedenheit zugeordnet, so muss die Reizverschiedenheit in dem physiologischen Geschehen irgendwie abgebildet sein, denn nur verschiedene physiologische Vorgänge können zu verschiedenen Verhaltensweisen führen. Ist in vergleichend psychologischen Untersuchungen auf makroskopischem Wege eine Zuordnung von Situation und Verhalten gefunden worden, so gehört zum vollen biologischen Verständnis das Auffinden des für die Situation spezifischen physiologischen Korrelats hinzu.

Eine allgemeine physiologische Theorie des Bewegungssehens muss in der Hauptsache drei Erscheinungsgruppen gerecht werden, die in vergleichenden Untersuchungen festgestellt worden sind.

Eine Zuordnung von Verhaltensweisen zu in ihrer Raumlage wechselnden Gegenständen erfolgt

(1) wenn eine Lichtverschiebung auf einem Bereich der Retina stattfindet (reelle Bewegung);

(2) wenn ein Bereich der Retina konstant bleibt im Verhältnis zur Lichtverschiebung eines umfassenden Bereichs (induzierte Bewegung);

(3) wenn getrennte Bereiche der (Wirbeltier-) Retina nacheinander einen Lichtwechsel durchmachen (kinematoskopische Bewegung).

Andere, mehr spezielle Bedingungen brauchen hier nicht berücksichtigt zu werden.

(1) DAS KORRELAT DES BEWEGUNGSSEHENS NACH HERTZ

Für den 1. Fall ist die Analyse des Reizgeschehens von Hertz (1934 a) anwendbar. Betrachten wir ein Sinnesfeld das aus einzelnen Sehelementen besteht, so lässt sich der Vorgang einer partiellen Lichtverschiebung, die einer Figur von der Grösse eines Elements entspricht, veranschaulichen als eine Folge von Erregungen, die immer auf benachbarte Sehelemente übergeht. In einer beliebigen Zeitphase ergibt sich folgende Lage. "Der Erregungskomplex, der der kleinen Figur entspricht, ist auf der Seite nach der die Bewegung fortschreitet, von einem Bereich konstant abweichender Erregung begrenzt, auf der anderen Seite aber von einem Bereich, der gerade selbst einen Erregungswechsel durchgemacht hat. Es besteht also für den Bereich der bewegten Figur und ihrer Umgebung in jedem Augenblick solange der Gegenstand in Bewegung ist, eine eigentümliche Schiefheit der Erregungslage, deren Asymmetrie uns die Richtung der stattfindenden Bewegung unmittelbar anzeigt, ohne dass wir dazu die verschiedenen Lagen des bewegten Gegenstandes in der Zeit zu vergleichen brauchten."

Wir sahen schon früher, dass ein Erregungszustand eines Sinneszellenbereichs nicht nur von der gegenwärtigen Reizung, sondern auch von der vorangegangenen Reizung und auch von dem Erregungszustand benachbarter Sinnesbereiche abhängt. Ähnlich haben wir auch in diesem Falle eine Funktion vor uns, die sich nicht in einer einzigen Nervenzelle oder einzigen Leitungsbahn entwickeln kann, sondern es gehören zu ihrer Charakterisierung als asymmetrischer Vorgang mindestens drei, in funktioneller Verbindung stehende Elementarorgane, in denen eine physiologische Struktur entsteht. So veranlasst also die reelle Bewegung auf die Retina projiziert keine blosse Ortsveränderung, sondern eine spezifische physiologische Funktion, die als Korrelat des Bewegungssehens anzusehen ist.

Auch für induzierte Bewegung (Fall 2) ist diese Anschauung anwendbar. Man muss nur annehmen, dass durch die Erregungsasymmetrie, die der tatsächlichen Lichtverschiebung in der Umgebung entspricht, in den Bereichen des Sehfeldes, in denen keine Verschiebung stattfindet, ebenfalls Erregungsasymmetrien hervorgerufen werden, etwa im komplementären Sinne. So wäre es möglich ohne Aenderung der Raumlage eines Objektes dieses bewegt zu sehen.

Ob eine Bewegung induziert wird, hängt von der figuralen Gliederung des Feldes ab, indem die phänomenale Bewegung "Verschiebung im natürlichen Bezugssystem" ist (Duncker, 1929). Das natürliche Bezugssystem wird von den Handlungsmöglichkeiten bestimmt, die die Umgebung für den Organismus zulässt. Mit Duncners Beispiel bezieht sich ein Knopf sinngemäss zu seinem Rock und nicht umgekehrt. Dass neben figuraler Gliederung auch die Fixation ein Faktor ist, der zur Bewegung des betreffenden Gegenstandes führt, wird verständlich wenn man bedenkt, dass die Fixation zur figuralen Gliederung beiträgt. Es werden aus biologischen Gründen eben diejenigen Stellen des Sehfeldes fixiert, die möglichst genau erfasst werden sollen, wobei es nicht nur auf das Scharfsehen, sondern auch auf die Ortsorientiertheit und Bewegtheit ankommt. Es kommt immer auf relative Verschiebungen, d. h. auf Abstandsänderungen an, denen man ein Geschehen zwischen den Reizkorrelaten der den Abstand ändernden Gegenstände im physiologischen Feld zugeordnet denken kann.

(2) HYPOTHESEN ÜBER KINEMATOSKOPIE (Köhler, Schiller)

Es bleibt noch übrig, die Erscheinungen der Kinematoskopie in Betracht zu ziehen (Fall 3). Nach Köhler (1923) erzeugt eine Lichtfigur im Sinnessektor einen zentralwärts steigenden Erregungsstrom, der nicht auf festgelegten Bahnen verläuft, sondern nach hydrodynamischen Modellen vorzustellen ist (Hartmann, 1923). Steigt ein zweiter Erregungsstrom zentralwärts, während der erste unterwegs ist, so kann die dadurch herbeigeführte Änderung in der dynamischen Kräfteverteilung im Felde zu einer Wechselwirkung beider Ströme führen. So ist z. B. eine gegenseitige Anziehung beider Strömungssäulen vorstellbar, so dass es unter geeigneten Bedingungen auch zur Vereinigung der beiden Strömungen kommen kann, wobei die bereits vollentwickelte erste, die erst ansetzende zweite an sich reisst. Diese Wechselwirkung nimmt ihren Anfang bevor die Ströme dasjenige Zentrum erreichen, wo das Geschehen einem Erleben entspricht. Ist in der ersten Phase der

Wechselwirkung die erste Strömung im Übergewicht, so dass die ihr entsprechende Figur an der ungefähr entsprechenden Raumstelle erscheint, so wird doch beim allmählichen Abklingen der ersten Erregung die zweite Strömung im Verhältnis allmählich stärker, so dass diese das Übergewicht erreicht und auf dem Zentrum, das dem Erleben entspricht, die mit ihr vereinigten Reste der ersten Strömung mit sich reisst ungefähr an die Stelle, die der zweiten Figur entspricht. Diesem Mitreissen entspricht die gesehene Bewegung einer einzigen Figur.

Ich möchte diese Hypothese etwas modifizieren um sie auch für die Erklärung der Alternativversuche tauglich zu machen. (Diese Vorstellungen sind andernorts nicht publiziert.) Das Wesentliche in den Alternativversuchen und in manchen Erscheinungen des reellen Bewegungssehens war darin zu erblicken, dass die zweite Figur noch garnicht zu sehen ist, während die erste sich schon in die betreffende Richtung bewegt. Der zweite Reiz zeigt also seine Wirkung schon bevor er zur Wahrnehmung führt. Das ist auch im Sukzessivvergleich der Fall. Ich will aus diesem Grund eine Art Vorwirkung des Erregungsvorgangs annehmen. Auch Köhler ist geneigt eine Vorwirkung anzunehmen, ohne aber im Wesen mehr zu sagen als folgt: "Ist also die Verschiebung des Prozesses... ein recht langsamer Vorgang... so steht dem wohl eine sehr viel schnellere Kraftausbreitung gegenüber, durch welche das Feld im ganzen konstituiert, seine Änderungen so gut wie ohne Zeitverlust von jedem Punkt wirksam gemacht und deshalb auch jene relativ langsamen Verschiebungen nach Gestaltprinzipien erzeugt werden." Dabei wird die Prozessverschiebung mit der Erregungsfortpflanzung, die feldkonstituierende Kraftausbreitung mit dem Elektrotonus der gereizten Nerven in Zusammenhang gebracht.

Köhlers Annahme reicht nicht aus für qualitative Verhältnisse, die keine Ortsverschiedenheit bedeuten, wie z. B. für die Farbwandlung in der Bewegung. Es genügt nicht von einer Anziehung und Vereinigung zu sprechen, sondern man muss auch die qualitativen Übergänge verständlich machen. Das geschieht wenn wir statt der Köhlerschen Annahme von Anziehung der Erregungsströme die Hypothese aufstellen, jeder Erregungsvorgang sende eine Vorwirkung ins Zentrum, die viel rascher eintrifft als die Erregungswelle, eine Vorwirkung, die mit den Eigenschaften des zugehörigen Erregungsvorgangs variiert, oder weniger genau, dieselben funktionellen Eigenschaften besitzt, wie die Erregung, und mit benachbarten Erregungsvorgängen auch in Wechselwirkung tritt, nur dass der Vorwirkung kein Erlebnis entspricht. Wir können zulassen, es entspricht jedem Punkt auf der Retina je ein Punkt in dem Sehzentrum (Sehrinde oder subkortikalen Zentrum oder optischen Ganglion) und die Nervenbahn führt bis dorthin vollkommen isoliert. Obwohl die Köhlersche Hypothese diese Vortstellung verwirft, werden, wie ich glaube, viele Histologen darauf bestehen wollen. Eine Wechselwirkung zwischen den Punkten soll sich nach meiner Vorstellung erst im Zentrum ergeben. Die erwähnte Vorwirkung ist nun so vorzustellen, dass im Augenblick der retinalen Reizung der Zustand der zugehörigen Leitungsbahn und der Zentrumpunkte verändert wird, noch bevor die Erregungswelle sich bis hinauf fortpflanzt. Im Augenblick der peripheren Reizung wird der gereizte Bereich bis hinauf zum

Sehzentrum nervenelektrotonisch affiziert, so dass irgendeine Wirkung der Reizung im Zentrum früher aufzufinden ist als der Erregungsvorgang selbst. Diese Vorwirkung ist erwiesenermassen real. Gildemeister u. Weiss (1903) liessen Reizwirkungen durch nachgesandte Polarisationsströme auslöschen; letztere müssen also erstere eingeholt haben. Ihre Versuche belehren "dass der physiologische Ausdruck des Elektrotonus, gleich dem physikalischen, eine unvergleichlich grössere Ausbreitungsgeschwindigkeit besitzt als die Erregungswelle". Die Vorwirkung in dem Sehzentrum soll "virtueller Vorgang" genannt und als Vorzeichen des Erregungsvorganges aufgefasst werden. Einfache Analogien zur Veranschaulichung: das Seil spannt sich aus und belastet den Hacken im Augenblick, wo der Turner zu steigen beginnt; oder: ein leuchtendes Geschoss beleuchtet die Bahn die es passieren wird.

Wie gesagt, können ein virtueller Vorgang und ein Erregungsvorgang wie zwei Erregungsvorgänge miteinander in Wechselwirkung treten, so dass ein Erregungsvorgang zu anderen Erlebnissen führt, wenn er mit benachbarten virtuellen in Beziehung kommt, als ohne solchen. Die Wechselwirkung erfolgt nach den Gesetzen dynamischer Kräfteverteilung, die nach dem Gestaltprinzip zu Gleichgewichtslagen tendiert. So erfolgt z. B. die Anziehung und Vereinigung zweier örtlich und zeitlich benachbarter Prozesse, wie überall im Physischen, bei sozusagen grösster Ökonomie durch geringste Umwandlungen; daher werden gleiche Teilvorgänge von gleichen und nicht von ungleichen angezogen, entsprechend der Verschmelzungstendenz gleichen und ähnlichen Nervengeschehens nach dem Gesetze von Ranschburg (1913). Bei optimalen kinematoskopischen Zeitverhältnissen erfolgt die Vereinigung vorhandener Erregungsvorgänge mit hinzukommenden virtuellen, die vom zweiten Reiz herrühren, gerade in der Zeit, bis der Erregungsvorgang vom zweiten Reiz her an die betreffenden Zentrumpunkte anlangt, wohin der virtuelle Vorgang den ersten Erregungsvorgang inzwischen "angezogen" hat (in räumlicher und in qualitativer Hinsicht). Die in meinen Alternativversuchen gefundenen optimalen Intervallzeiten von 10–25 Sigma entsprechen nun tatsächlich in der Grössenordnung der vermutlichen Dauer der Erregungsfortpflanzung, auf die wir bei der Behandlung der Latenzzeit zu sprechen kamen.

Bei dem Sehen von physikalisch stetigen Bewegungen ist das physiologische Geschehen dasselbe, nur wird der Erregungsvorgang hierbei von immerfort vorangehenden virtuellen sozusagen gelenkt. Auf Grund dieser Vorstellung ist es verständlich—falls man mit der Gestalttheorie im Zentralfeld dem Reizfeld entsprechende räumliche Lagerung voraussetzt—dass beim Fröhlich'schen Phänomen die Verschiebung des bewegten Striches von dem *Ende* der Bahn abhängt. Hört nämlich fast gleichzeitig mit dem Verschwinden des Reizes die nerventonische Affizierung an Stellen auf, die in der Bewegungsrichtung vor den Stellen sind, auf welchen das der gesehenen Bewegung entsprechende Korrelat sich befindet, so beeinflusst keine Anziehung der (nicht mehr vorhandenen) virtuellen Vorgänge die Lagerung des Korrelats, die bei vorhandener Wechselwirkung den Strich in der Bewegungsrichtung verlagert erscheinen liesse, und zwar umso mehr, je länger die Bahn, d. h. an je grösserem Bereich der Bahn Vorwirkungen vorhanden

sein können. Auch das Abrundungsphänomen Rubins (1932) erklärt sich aus unserer Vorstellung. Die vom Punkt nach der Knickstelle entsandte Vorwirkung zieht den Erregungsvorgang der gleichzeitig im Zentrum einer früheren Stelle entsprechend sich befindet, in ihre Richtung an, so dass auf das Korrelat des erlebten Punktes zwei Kräfte einwirken: (1) die des auf gerader Bahn weiterschreit-

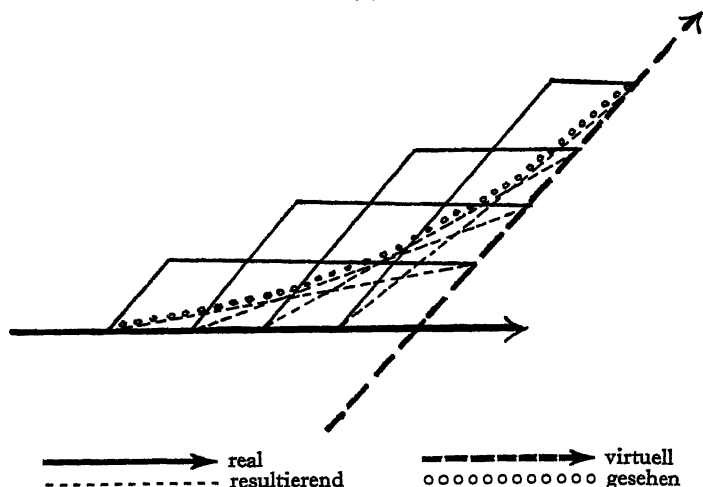


Fig. 11. Veranschaulichung des Zusammenwirkens zweier Faktoren beim physiologischen Prozess des Bewegungssehens. Vgl. Text.

enden Erregungsvorganges, (2) des schräg schreitenden virtuellen Vorgangs, die—da Anfangs die gerade Erregung, später bei Annäherung dieser an die Knickstelle die schräge Komponente überwiegt—in einer Bewegung auf bogenförmigem Verbindungswege resultieren (vgl. Fig. 11, die ausser für örtliche (wie in Fig. 4) auch für figurale und Farbumwandlung Darstellungswert hat, bei entsprechender Auslegung der Komponentensymbole). So versteht sich z. B. die Beobachtung von Higginson (1926 b) dass der Farbwandel in der Kinematoskopie um die Mitte der Bahn stattfindet.

(3) GLIEDERUNG DES REIZFELDES UND AUFLÖSUNGSVERMÖGEN IM REZEPTOR

Sukzessive diskrete Reize, die in grösseren Zeitabständen aufeinanderfolgen, werden von der Wechselwirkung weniger tangiert, aber als solche werden sie nur dann perzipiert, wenn sie in ganz grossem Zeit- (und Qualitäts-) abstand auftreten. Folgen zwei nicht sehr verschiedene Reize während reizarmen Geschehens in wenigen Sekunden aufeinander, so erleben wir einen Übergang zwischen beiden, in der die Figuren zwar nicht gesehen werden, aber ihre Beziehung ist anschaulich gegeben, in einem Wachsen, Hellerwerden, usw. (sinnliche Relationserfassung, Sukzessivvergleich). Ist die Intervallzeit nur ein Bruchteil der (nicht zu langen) Reizdauer, so erlebt man Bewegung oder Verwandlung einer Figur, bei noch kleineren ein gleichzeitiges Vorhandensein beider Reizglieder. Bei rascher periodischer Abfolge erscheinen die Reize in Einheit verschmolzen, bei etwas geringerer

Geschwindigkeit entsteht Flimmern und bei noch geringerer Bewegung, bis bei ganz geringer, abwechselnde Reizglieder wahrzunehmen sind.

Verschmelzen, Flimmern, Bewegung und Verwandlung, Sukzessivvergleich von Paaren, das sind qualitative Varianten der Wahrnehmung, die bei quantitativer Veränderung eines physiologischen Geschehens auftreten. Das quantitative Mass dieses Geschehens ist von zwei Faktorengruppen abhängig.

(1) Von der Gliederung des physikalischen Geschehens in der Umgebung eines Organismus (Reizfeld).

(2) Von der Empfindlichkeit der Rezeptoren dieses Organismus (Sinnesfeld).

Die gleiche Wahrnehmungsvariante kann entstehen wenn das Reizfeld stärker gegliedert wird (innerhalb eines Zeitabschnitts und einer Fläche) bei konstantem Sinnesfeld oder wenn bei konstantem Reizfeld ein weniger feines Sinnesfeld herangezogen wird. So ergibt sich einerseits, dass bei demselben Organismus im Falle einer raschen Aufeinanderfolge Verschmelzung eintritt, bei langsamerer Bewegung und bei weiterer Verlangsamung sukzessives Paarerlebnis, andererseits aber bei konstanter zeitlicher Gliederung des Reizfeldes der Mensch Verschmelzung sieht, der Kampffisch aber Bewegung. Diesem Unterschied in dem zeitlichen Auflösungsvermögen steht eine Wahrnehmungsvariation, die von dem räumlichen Auflösen abhängt, zur Seite. Ein Schema soll diese Verhältnisse veranschaulichen, das sowohl für zeitliche, wie für räumliche und qualitative Verhältnisse übertragbar ist (Fig. 12).

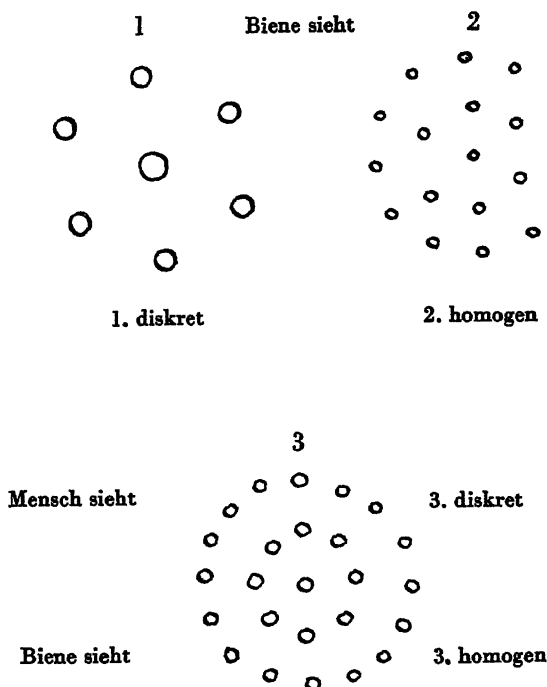


Fig. 12. Diskrete Reize werden wahrgenommen wenn (obere Figur) bei konstantem Rezeptor die Einzelreize weit auseinanderliegen (1) oder wenn (untere Figur) bei konstanter Reizdichte das Auflösungsvermögen des Rezeptors fein ist (Mensch).

Es wird in der Verschiedenheit der Auflösungsverhältnisse der Arthropoden gegenüber den Wirbeltierrezeptoren liegen, dass für letzteres ein grobgliederter Vorgang genügt (kinematoskopisches Reizfeld), wo ersteres kontinuierlichen physikalischen Vorgang erfordert um das physiologische Korrelat hervorzurufen, das für die biologische Leistung der Bewegungswahrnehmung verantwortlich ist.

V. ZUSAMMENFASSUNG

Berichtet wird über Phänomene des Bewegungssehens die an Menschen und Tieren festgestellt sind. Visuell wahrgenommene Bewegung liegt vor, wenn einem optischen Wechsel in der Umgebung, bei alleiniger Vermittlung des Lichtes, eine Reaktion entspricht, die je nach dem Stand dieses Wechsels spezifisch gerichtet ist.

Verschiedene Sinneselemente machen infolge geometrischer Veränderungen in der Umgebung einen Belichtungswechsel durch, so dass an Stelle einer objektiv kontinuierlichen Verschiebung eine Folge von Erregungen im Rezeptor auftritt (reelle Bewegung). Werden dabei einige Elemente, die in den Bereich der Erregungsverschiebungsbahn fallen, übersprungen, so ändert das unter gewissen Bedingungen nichts am Effekt (Kinematoskopie). Bleibt ein Bereich in einem umfassenden optischen Wechsel konstant, so erfolgt die Reaktion oft so als ob der konstante Bereich sich verschoben hätte und der umfassende Wechsel konstant geblieben wäre (induzierte Bewegung).

Bewegungssehen unter experimentellen Bedingungen an Tieren hat zuerst Doflein an Dekapoden nachgewiesen. Wird die Umgebung optisch verschoben, so treten optomotorische Reaktionen auf, die ausser von Wirbeltieren auch von höheren Insekten ausgeführt werden, nicht aber von Coccinellen, die nur Lagekorrekturen ausführen (Gaffron).

Wolf stellt die optomotorischen Reaktionen an Bienen fest und weist nach, dass Bienen schnelleren Reizwechsel dem langsameren vorziehen. Hertz erbringt den Nachweis, dass diese "Flimmertaxis" nicht als Grundlage des Bewegungs- und Formsehen anzusehen ist, wie Wolf annimmt. Fliegen können ruhende und bewegte Muster auch dann unterscheiden, wenn erstere infolge der Körperbewegung des Tieres ebenfalls eine Lichtverschiebung auf dem Lichtsinnesorgan hervorrufen.

Bewegungsschwellenbestimmungen wurden ausser an Arthropoden auch an Hühnern und an Ratten ausgeführt. Uexkülls Schule hat die Verschmelzungsgrenzen und die Schwellen für langsamste und schnellste Bewegung am Kampffisch *Betta* festgestellt und nachgewiesen, dass eine Bewegung für *Betta* ungefähr zweimal so schnell erscheint wie für den Menschen. Es besteht also ein Unterschied in dem zeitlichen Auflösungsvermögen der verschiedenen Organismen. Dieses Auflösungsvermögen ist von der Chronaxie, der Refraktärphase und der Latenzzeit abhängig.

Die kinematoskopische Bewegung ist von der realen nicht unterscheidbar (v. d. Waals u. Roelofs). Sie soll nach der Produktionstheorie durch die Phantasie entstehen. Unter den Beweisen, die für eine physiologische Entstehung des Phänomens

sprechen, nehmen die Alternativversuche eine wichtige Stellung ein. In Situationen die zwei Bahnen ermöglichen, lässt sich auf Grund einfacher Figuralgesetze voraussagen welche eintreten wird (Schiller). Die Kinematoskopie ist auch für Wirbeltiere nachweisbar, wie das Gaffron mit der optomotorischen Reaktion und Schiller mit Dressurversuchen an *Phoxinus* gezeigt haben. Die genannten Forscher weisen aber mit verschiedenen Methoden nach, dass Arthropoden (*Aeschna*, bezw. *Galathea*) kein kinematoskopisches Sehen besitzen. Das Komplexauge ist also für die Organisation von Bewegungen aus diskreten Reizen nicht geeignet.

Die induzierte Bewegung lässt diejenige Raumstelle bewegt erscheinen, die in einem Bezugssystem lokalisiert ist (Duncker). Im Falle einer umfassenden Bewegung der Umgebung wird der Körper des Beobachters als bewegt erlebt. Gaffron weist die induzierte Bewegung bei *Aeschna* nach, indem dieses nur auf bewegte Objekte jagende Tier die Fangreaktion ausübt wenn hinter einem ruhenden Fleck, auf den es sonst nicht reagiert, ein Streifenmuster vorbeigezogen wird.

Unter den physiologischen Theorien sind die von Hertz und von Köhler für reelle und induzierte bezw. für kinematoskopische Bewegung behandelt. Nach Hertz ist das physiologische Korrelat des Bewegungssehens in der Erregungsasymmetrie zu suchen, die während des Erregungsfortschreitens im Sinnesfeld entsteht. Nach Köhler besteht eine gegenseitige Anziehung der Erregungsströme bei diskreter Reizlage, die für das einheitliche Sehen der Bewegung verantwortlich ist. Verfasser modifiziert diese Hypothese in dem er einen tonischen Vormeldungsprozess annimmt, der die Entwicklung des vorangegangenen Erregungsvorganges beeinflusst. Diese Annahme wird an den Alternativversuchen und an von Rubin entdeckten Phänomenen antizipatorischer Art veranschaulicht.

Je nach der zeitlichen und räumlichen Gliederung des Umfeldes und je nach den Auflösungsverhältnissen der Sinnesorgane—sowohl in der bekannten räumlichen, wie in der hier entwickelten zeitlichen Hinsicht—entsteht entweder die Wahrnehmung von diskreten, oder von einheitlichen Vorgängen.

VI. SUMMARY

Movement-vision (i.e. the visual perception of changes in the geometrical arrangement of the light pattern of the environment) in man and animals has been dealt with in this review.

A change in the geometrical arrangement of the light pattern is said to be visually perceived when it calls forth a reaction which is determined in its direction by the nature of the change.

In *real movement-vision* a number of sensory elements in an eye, as the result of the pattern changes in the surroundings, undergo a change in illumination, and, in consequence of the mosaic structure of the retina, there arises in it a series of discrete excitations. If certain sensory elements of the retina which fall in the path of the moving excitation are not stimulated, and the animal nevertheless reacts as in the case of real movement-vision, we then speak of *kinematoscopy*. If a certain restricted region of a field of vision does not move when the rest of it is in motion, an animal often reacts as if the stationary region were moving while the part of the field actually in motion remained stationary: this is spoken of as *induced movement-vision*.

Movement-vision was first experimentally demonstrated by Doflein in decapod crus-

taceans: if the surroundings move, optokinetic reactions ensue. Such reactions also occur in vertebrates and insects, although coccinellids only react by changes in posture.

Wolf demonstrated optokinetic reactions in bees. He also showed that these animals respond better to quick than to slow changes of stimulus. Hertz proved that this so-called "flickertaxis" is not the basis of movement-vision and form-vision, as Wolf assumed. Flies can distinguish stationary from moving patterns, even when the former produce a displacement of light on the retina as a result of the animal's own bodily movement.

Thresholds for the perception of movement have been studied in arthropods, fowls and rats. Experiments by Uexküll's school have shown that a given movement would appear about twice as quick to the fighting fish, *Betta*, as it does to man. Thus the resolving power for consecutive optical stimuli varies in different animals. This temporal resolution depends on chronaxie, refractory period and latent period.

Kinematoscopic movement cannot be distinguished by the animal from real movement. According to the "Produktionstheorie" the apparent identity of the two is due to the imagination. Amongst the proofs of a physiological origin of the phenomenon, experiments with alternating images take a prominent place. When it is possible for an image to follow one of two virtual paths, it can be predicted by simple rules which path will be adopted. Kinematoscopy has also been demonstrated in vertebrates by optokinetic reactions and by training experiments. It has been shown, however, by various methods, that arthropods have no such kinematoscopic vision. Thus the compound eye is not suitable for the synthesis of movements from discrete stimuli.

Induced movement-vision causes a stationary region in space to appear to be in motion, whenever that region assumes a certain importance relative to its moving surroundings; when a considerable portion of the surroundings is in motion, an observer has the sensation of being in motion himself. It has been shown that a similar induced movement-vision exists in the dragon-fly, *Aeschna*, which hunts moving prey; if a striped pattern is moved behind a stationary spot, the latter calls forth the animal's snapping reflex.

Amongst the physiological theories of real, induced, and kinematoscopic movement-vision, those of Hertz and Köhler have been reviewed. According to Hertz, the physiological explanation of movement-vision is to be sought in asymmetry of stimulation arising during the progression of the stimulation across the sensory field. According to Köhler a mutual attraction between streams of excitation arising from two discrete points of stimulation is responsible for uniform movement-vision. The author has modified Köhler's hypothesis by assuming a tonic heralding process influencing the effect of preceding excitation. This assumption is supported by experiments with alternating images and by other anticipatory phenomena discovered by Rubin.

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NOTE ON THE PHYLOGENY OF FOSSIL CEPHALOPODS

By L. F. SPATH

(Received 25 October 1936)

THE problem of the evolution of the Cephalopoda was discussed at some length in vol. 8 of *Biological Reviews* (1933). If such things could be proved there would, of course, have been no need to restate the problem; and many think that it is still unsolved. But the conclusions arrived at in my article were too briefly, and perhaps too bluntly, summarized, and they invited criticism. Thus Prof. Schindewolf, in a note entitled "Concerning the evolution of the Cephalopoda" and published in vol. 9 of *Biological Reviews* (1934), largely disagreed with my views. He also announced the forthcoming publication of two larger papers on the subject, and these, of course, required a full reply. This reply, belated, but not through my fault, is now in the press. Thanks largely to the kind intervention of Prof. Schindewolf himself, my reply is being published in the November number (Heft 3) of the *Palaeontologische Zeitschrift*, a publication in which Prof. Schindewolf's original paper on the phylogeny of the Ammonoidea appeared.

I wish to direct the attention of readers interested in this research to the reply to my critics. I will confine myself to quoting the summary and to stating that my reply is not only a reaffirmation of the views put forward in my first article in *Biological Reviews*. It was not necessary for me to review my material and to make exhaustive new investigations; but there were several points on which I had not made myself quite clear, and I am glad of the opportunity to reply to my critics in detail. Some illustrations published by Prof. Schindewolf also helped in the discussion, and a plate is now included in my reply, giving micro-sections that show the wandering siphuncle on the early whorls of *Dimorphoceras* and *Gastrioceras*.

The summary is as follows:

(1) This paper is both a sequel to my first review, necessitated by the rapid advance in this line of research, and a reply to the criticisms of Schindewolf, George, Schuchert, Teichert, Ulrich and Foerste, and others.

(2) The principal conclusions previously arrived at with regard to the phylogeny of the Nautiloidea, the derivation of the goniatites, and the origin of the later ammonoids are not considered to have been shaken, and additional evidence is offered. The errors of which I have been accused are also discussed.

(3) The genus *Volborthella* remains as doubtful as before. The earliest cephalopods known are cyrtocoines.

(4) Various features that had not previously been mentioned are discussed, from the nucleus of the completely imperforate Lower Ordovician *Trocholites* to the

hyponomic sinus of the early goniatites. The range in time of the latter is also taken to confirm my views.

(5) Additional evidence is offered to show the instability of the siphuncle in the goniatites (Pl. IX).

(6) The advantages of an elastic classification are discussed, also some aspects of recapitulation.

With regard to the genus *Salterella* which had previously been mentioned and which is held by some to be a cephalopod, readers of *Biological Reviews* who are interested may like to know that a paper by me, entitled "So-called *Salterella* from the Cambrian of Australia", appeared in the *Geological Magazine* for October 1936, and discussed the problematical nature of this organism.

THE INTERPRETATION OF THE FLOWER: A STUDY OF SOME ASPECTS OF MORPHOLOGICAL THOUGHT

By AGNES ARBER

(Leverhulme Research Fellow, Cambridge)

(Received 10 February 1936)

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I. INTRODUCTION

SINCE 1790, three phases have succeeded one another in the interpretation of the flower; they illustrate the swing of the pendulum so often observed in the history of scientific theories. The first phase was that of the pre-Darwinian morphology associated with the names of Goethe and de Candolle; the second was that of post-Darwinian phylogenetic morphology; while the third is represented by certain recent developments, in which a phylogenetic aim has been discarded, and a return has been made to a standpoint not far removed from that of Goethe. These three phases overlap, however, to a considerable extent: the second, especially—that of phylogenetics—still exists to-day, side by side with more modern approaches to the subject.

This three-phase historical sequence provides the thread running through the following pages, and only those aspects of floral morphology are considered which have some direct bearing—affirmative or negative—upon the stream of thought initiated by Goethe. The present survey thus covers only a strictly limited field, and various important parts of the study of the flower are deliberately left untouched.

II. THE FLOWER AND THE VEGETATIVE SHOOT

The view of the flower proposed by Goethe (1790) is that it is equivalent¹ to an abbreviated vegetative shoot, while the individual floral members are equivalent to foliage leaves. This theory, which places vegetative and reproductive structures in the same general category, cannot well be harmonized with the analytical trend which took its origin in the nineteenth century and is still conspicuous in the work of certain writers of the present day. This analytical bias is shown, for example, by Zimmermann (1930), who defines morphology as that science which, in contrast to systematics, is concerned, not with the plant as a whole, but with its parts—organs, tissues, and so forth. It is difficult to see how so narrow a definition can be justified. The Aristotelian conception of form comprehended “organization, the relation of part to part, and the subordination of all parts to the whole” (Stace, 1920); the study of form to-day should have no less a content. Morphology on this wider view may perhaps be considered as the study of the plant as a whole, under the aspect of form—*sub specie formae*—the word “form” being used, not only in its more obvious meaning, but also to include the entire organization, external and internal, from the beginning to the end of the life history. Goethe’s view is broadly consistent with this holistic conception of morphology, since it recognizes a common basic scheme of organization underlying both the vegetative and reproductive systems. His general standpoint seems to be justified by the fact that the flowering phase is not an isolated event, but is heralded and accompanied by changes in the vegetative parts; the phyllotaxis, for instance, may be altered, and there may be a gradual replacement of elaborate foliage leaves by other leaf members of a simpler type. Moreover, the reproductive shoots often closely resemble the vegetative shoots of the same plant in their general habit and mode of branching. For example, the repetitive branching, which is characteristic of certain vegetative shoots, can be traced also in the corresponding inflorescences; the “tillering” and “pompon” growth of certain grasses is a case in point, for it is exactly equivalent to the clustered inflorescence development in the same family (Arber, 1934, pp. 215, 261–2). The impression left on one’s mind by such facts, and also by the detailed study of anthocladia and inflorescences made by Goebel (1931), is that reproduction ought to be treated as a function of the entire plant, and that the reproductive shoot cannot be understood except in relation to the vegetative body. Such studies as those of Salisbury (1931) upon *Ranunculus parviflorus* L., and of Marsden-Jones (1935) upon *R. Ficaria* L.—in which the flower is treated in conjunction with the vegetative structure and the life history—illustrate the advantages of this holistic approach.

When we pass from the reproductive phase in general to the detailed comparison of the vegetative shoot and the flower, we find that they each arise in the axil of a member, which is often indisputably a leaf, whether it is called a foliage leaf or a bract. Moreover, they each consist of an axis of similar structure, and they bear

¹ In the earlier part of the present article, “equivalence” will be taken at its face value; the actual meaning to be attributed to it is considered in pp. 172–174.

first leaves, which, though they happen to be called "prophylls" in the vegetative shoot and "bracteoles" in the flower, show an evident correspondence. In the monocotyledons, where one adaxial prophyll is the rule, we also find one adaxial bracteole, while in the dicotyledons, where paired prophylls are placed to right and left, there are, correspondingly, paired bracteoles similarly placed. It seems then that, in the architecture of the system of which it forms a part, in its individual position, and in its first leaves, the flower resembles a vegetative shoot; it is when the region above the prophylls or bracteoles is considered, that the divergences between the vegetative shoot and the flower become more evident. The primary divergence is that the axis of the vegetative shoot tends to elongate, so that the leaves are separated by well-defined internodes, whereas the floral axis, though it often elongates conspicuously *below* the flower, tends to be telescoped from the level of the lowest sepal. This difference is a very general one, so much so that it is not easy to point to any purely vegetative shoot bearing normal leaves which remains *permanently* unextended. It thus seems that internodal suppression is definitely correlated with the reproductive character. Goethe¹ (1790, § 114) understood this distinction. He wrote: "A plant *vegetates*, spreads itself more or less, and develops a stalk or stem; the intervals from node to node are generally noticeable, On the other hand, a plant which *flowers* has contracted all its parts, . . . and all the organs are in a highly condensed state, and developed in close proximity to one another." Owing to this contraction, the best term of comparison for the flower is not a mature vegetative shoot, but a vegetative bud; the flower might indeed be described as corresponding to a vegetative shoot which has remained in a condition of permanent infantilism. It is widely recognized that a retention of infantile characters has sometimes played a part in evolutionary history, and it is not inconceivable that something of the kind may also occur in the ontogeny of the individual.

We are on firmer ground when we leave such speculations and return to the *fact* of the abbreviation of the floral axis, and to certain consequences of this abbreviation, which reduce the resemblance to the vegetative shoot. One of these consequences is that the normal order of development of lateral appendages (older below, younger above) is sometimes affected in the flower by the precocious appearance of those nearer the apex (Goebel, 1933, p. 1837). In other cases the checking of apical elongation may bring about a change in the general form of the flower, since growth, and appendage-forming activity, may be transferred from the centre to the margin of the receptacle, and perigyny or epigyny may be the outcome.

A further result of the early loss of vigour shown by the growing point of the floral axis, is that it may be wholly used up in the formation of the ultimate appendages (carpels). This divergence has been used by Grégoire (1931) as evidence for his contention that Goethe's view was wrong, and that the flower and vegetative shoot belong to categories which are fundamentally different. He states that in the flower the tip of the axis is always entirely used up to form carpels, no residual

¹ Throughout this article, the quotations from Goethe (1790) are taken from the writer's own unpublished translation of that essay.

apex being left over, whereas he considers that this is never true of the vegetative shoot; but both these statements need qualification. His remarks about the Primulaceae may be taken as an example. He writes that in this family, "le fait que des ovules se forment sur le sommet de la colonne centrale suffit à montrer que celle-ci ne représente pas un cône végétatif persistant". Van Tieghem, however (1871, p. 90, Pl. XV, fig. 489), long ago figured a radial section of a gynaeceum of *Primula sinensis* in which he showed the naked axis of the column extending above the ovules, and he described this column as having a conical termination, "dont la pointe s'enfonce dans la base du style sans jamais y adhérer". Moreover, the same thing was described at a later date for several members of the family by Vidal (1900), who commented on "la longueur véritablement extraordinaire qu'a l'axe floral au-dessus de la région ovulifère chez les Primulacées". Since these observations are of early date, it seemed that the apex of the floral axis in this family ought to be re-examined with more modern technique; so I have cut microtome series through the flowers of *Primula vulgaris* Huds., *P. farinosa* L. and *Anagallis arvensis* L. I have found that my sections completely confirm the descriptions of van Tieghem and Vidal, for in all three species the column, on which the ovules are borne, is seen to be prolonged centrally above them as a minute naked tip. It must be remembered, however, that the nature of the "free central" placenta in this family is still a matter of controversy, so one ought not, perhaps, to take it absolutely for granted that the naked tip is merely the apex of the axis. An example of which the interpretation is less open to doubt has been recorded quite recently by Newman (1936). He shows from a study of serial sections through the developing flower of two species of *Acacia*, that the single carpel, which at maturity appears to be terminal, is, in origin, lateral to a minute residual apex, and that the terminal position which it ultimately assumes is a secondary effect. Other instances which might be cited are the residual apex of *Clematis recta* L. (Fig. 2, A₁), and of *Romneya* (Dickson, 1935, fig. 11 e, p. 187). It is possible, however, that none of these examples would be convincing from Grégoire's standpoint, because, though he is willing in some cases to admit the existence of an *apparent* apex above the carpels, he considers that this is never genuinely axial. For instance, he figures sections of *Aquilegia*, in which he shows a circle of five carpels, which have all the appearance of being placed laterally to a central axial cone. Nevertheless, he argues that this cone consists in reality of tissue which is not axial, but which would have formed a sixth carpel, if it had not been inadequate in amount for this purpose. This ingenious argument is from its nature difficult to refute; but in searching among apocarpous gynaecea for evidence for or against Grégoire's theory, I have found, in a bud of *Ranunculus acris* L., an example which seems to be crucial. Here the receptacle in the gynaeceum region continues as a minute cone above the base of the uppermost achene (Fig. 1, 1). This cone, on Grégoire's theory, would not be the end of the axis, but would consist of carpellary tissue. It happens, however, in this buttercup that the tissue of the floral axis is strikingly different from that of the young achenes, in the size of the elements and in the staining capacity of their cell walls; and the residual apical cone (Fig. 1, 2) is

so completely like the axis in the character of its tissues as to leave no doubt that it is in reality what it is in external appearance—the continuation of the axis upon which the achenes are borne laterally.

The impassable barrier which Grégoire fixes between the flower and the vegetative shoot depends not only on his contention that the apical regions of floral axes are invariably used up, in their entirety, in the formation of carpels, but also on his converse statement, that no vegetative axis ever has its apex entirely used up in the formation of leaves. It is admittedly a truism that in vegetative shoots of unlimited growth, the apex of the axis must have its existence continued inde-

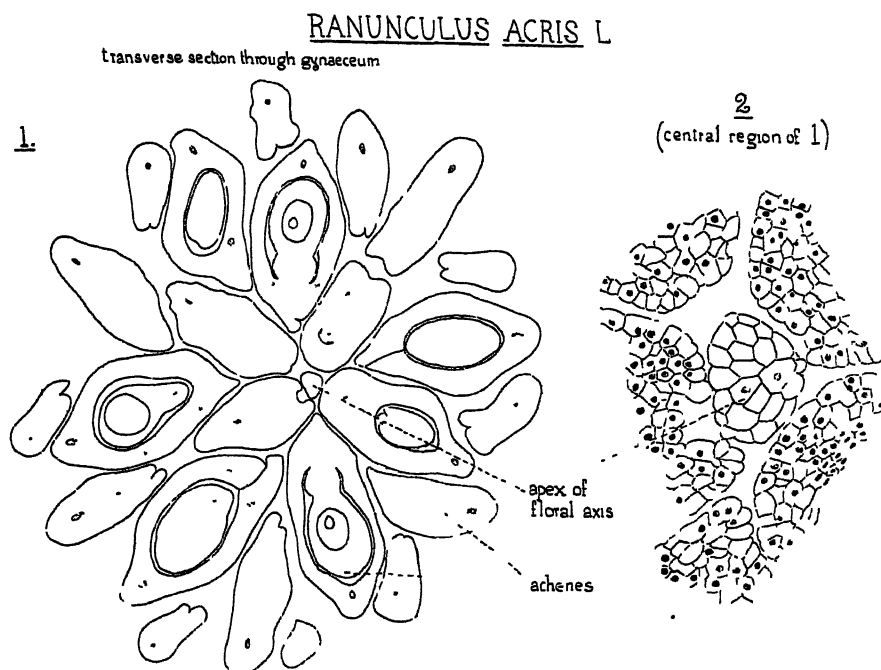


Fig. 1. *Ranunculus acris* L. 1, transverse section of young gynaecium to show residual apex ($\times 47$). 2, central region of 1 to show residual apex and edges of surrounding carpels on a larger scale ($\times 193$ circa).

finitely. It is not, however, to such shoots that we must look for the closest analogy with the flower, but rather to shoots in which a definite limit is set to elongation. Such an example may be found in *Asparagus Sprengeri* Reg., in which the vegetative shoot consists of an axis bearing short shoots in the axils of leaves. The ultimate and penultimate leaves, and the short shoots which they bear in their axils, may entirely use up the shoot apex, so that no residual trace is left between them (Arber, 1935, Fig. 1, B₁, p. 339; leaves 16 and 17, with the axillary bud between them in Fig. 2, 8, p. 340). Examples such as these are apparently not numerous among purely vegetative shoots, but they are less infrequent if inflorescences are included in the survey. I think that the significance of inflorescences, as furnishing types of structure intermediate between flowers and purely vegetative shoots, has not

hitherto been sufficiently stressed in morphological argument. It is generally agreed that inflorescence axes are morphologically equivalent to vegetative axes, and bracts to foliage leaves. Now I have shown elsewhere that the whole apex of the spikelet (partial inflorescence) in certain bamboos (Arber, 1928 *a*, Fig. 5, p. 185; 1930, pp. 301-2) and also the apex of the raceme in the Fumariaceae (Arber, 1931 *b*, pp. 341-2) may be transformed into a bract. Moreover, the principle is exactly the same in those plants in which the apex of an inflorescence axis is entirely divided between two bracts, each with a flower in its axil (Arber, 1931 *b*, Fig. 13, B₁, B₂, p. 341). The conclusion seems to be that, as soon as limitation of growth becomes a factor, there is nothing to prevent a leaf or leaves terminating a shoot; and that, though this happens most frequently in floral shoots (flowers), it is not confined to them.

It is not only when the question of terminal appendages is under discussion that the analogy of the inflorescence is useful. We may recall the comparison with the flattened and expanded capitulum axis of the Compositae, which may be regarded as an intermediate term between the floral receptacle and the elongated conical apex of a typical vegetative shoot.

III. THE FOLIAR INTERPRETATION OF BRACT AND SEPAL

The evidence discussed in the preceding section seems to favour the idea that the *axes* of floral and vegetative shoots are equivalent, but the question becomes more debatable when we turn to the *appendages* borne by these axes above the bracteoles or prophylls. That sepals and some perianth members can, in a broad sense, be equated with bracts, and bracts with foliage leaves, is, however, generally conceded. It is impossible, for instance, to draw a sharp line between bracts and perianth members in *Chimonanthus fragrans* Benth., while Salisbury (1931) has brought forward both morphological and physiological evidence for the equivalence of foliage leaves and sepals in *Ranunculus parviflorus* L. In the Nyctaginaceae, the distinction between an involucre of bracts and a calyx can scarcely be said to exist; the involucre enclosing a group of flowers in *Mirabilis multiflora* A. Gray appears to correspond exactly to the structure which would be regarded as a calyx in *M. jalapa* L., if this species were known only in isolation from the rest of the family. The relation of foliage leaf, bract, calyx and corolla, has recently been analysed for *Primula sinensis* by Anderson & de Winton (1935), by the method of comparing their reactions to mutant genes. The result of this study has been to show that there is a specially close correlation between the reactions of bract and calyx.

IV. THE FOLIAR INTERPRETATION OF THE PETAL

When we pass from the calyx to the corolla, the justice of the comparison with the foliage leaf is less obvious, but, in their dorsiventrality and laminar form, petals may be held to show some resemblance to reduced foliage leaves in which the differentiation of epidermis, mesophyll, and vascular system has remained at a

low level. It is possible that certain features, such as colour, scent, fragility, and low anatomical differentiation, in which petals are unlike foliage leaves, may be direct results of some chemical disturbance marking the transition between the vegetative and reproductive regions of the flower. A glimmer of truth may be foreshadowed in a remark made by Goethe (1790, § 45) that "it is a very probable theory that the colour and scent of petals are to be attributed to the presence in them of the male fertilising substance". The essential oils which give rise to flower scents may perhaps be regarded as the waste products of decomposition, which are likely to be harmful to protoplasm (Hampton, 1925), so that, conceivably, the ephemeral character of petals is due to a kind of self-poisoning.

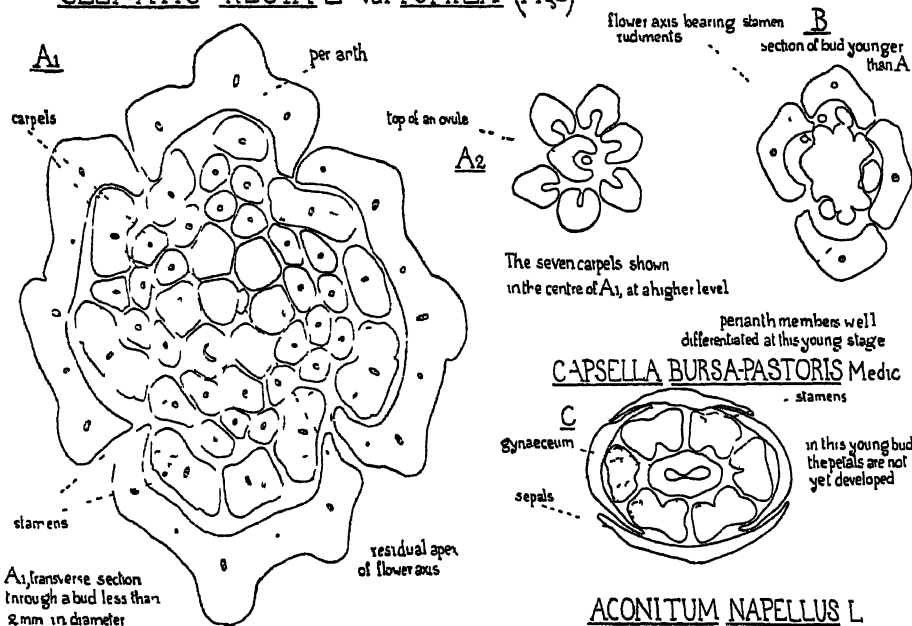
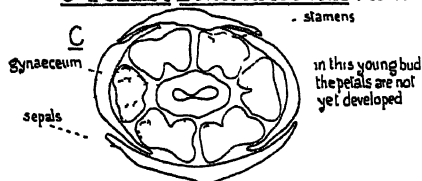
V. PETALS AND STAMENS

Goethe (1790) and de Candolle (1813) held the opinion that the corolla was of staminal nature. A good deal of evidence favouring this view has since accrued; this evidence is discussed in detail by Troll (1928) and Goebel (1933), and it will suffice here to call attention merely to a few of the points involved. Goebel connects the relatively late appearance of the petals in the ontogeny of the flower with their status as arrested stamens. This point is illustrated in Fig. 2, C, a section of a young bud of *Capsella Bursa-pastoris* Medic. Here, though the calyx is well developed, and the archesporium of the anthers is already formed, the petals have not yet appeared. As a contrasting example, the buds of *Clematis recta* L., drawn in Fig. 2, A₁ and B, may be noticed. In the Ranunculaceae, the perianth is generally admitted to be of the nature of a calyx, and, in agreement with this, we find that—unlike the corolla of *Capsella*—the perianth is well advanced even in such a section as that drawn in Fig. 2, B, from a bud so young that the other parts of the flower are quite rudimentary. Another fact which emphasizes the relation of petals and stamens is that in gynodioecism the absence of stamens tends to be associated with a relatively poor development of the corolla (Troll, 1928; Correns, 1907; etc.). For certain tribes of the Leguminosae, again, McLean Thompson (1924) has provided a delicate demonstration of the interdependence of stamens and petals.

Normal stamens as a rule show little resemblance to foliage leaves, except in arising laterally from an axis, and in being dorsiventral and bilaterally symmetrical. If, however, we accept the view that they are of the same status as petals, evidence pointing to the foliar character of the corolla will apply to the androecium also.

VI. THE FOLIAR INTERPRETATION OF THE CARPEL

The foliar theory of the carpel was put forward by Goethe (1790, § 78) in an incomplete form. He recognized that the legume was equivalent to a single folded leaf concrescent by its margins, and he was also clear about that type of syncarpous gynaeceum in which the carpellary "leaves" are united edge to edge; but he had not enough botanical knowledge to develop his views, and it was A. P. de Candolle (1827) who put the foliar interpretation on to a firmer basis. A recent statement of the theory, which is, however, too literal in its mode of expression (see p. 173),

CLEMATIS RECTA L. var PUMILA (A & B)CAPSELLA BURSA-PASTORIS MedicACONITUM NAPELLUS L.

(young gynaeceum)

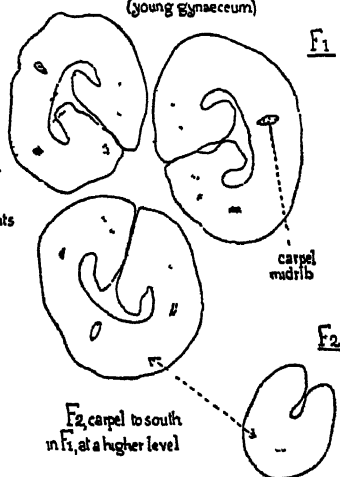
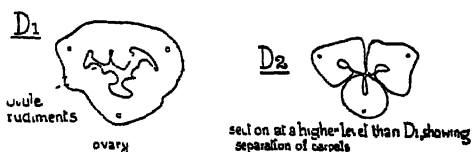
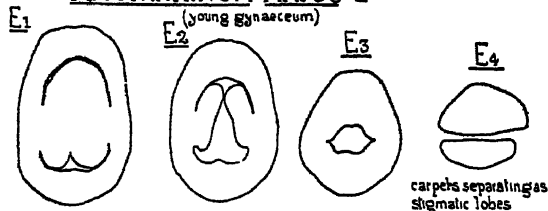
ASPARAGUS OFFICINALIS L.ANTIRRHINUM MAJUS L.

Fig. 2 All the diagrams from transverse microtome series from below upwards through young buds. A, B, *Clematis recta* L. var. *pumila* (Cambridge Bot. Gard.). Sections ($\times 47$). A₁, young bud; A₂, gynaeceum only of the same bud at a higher level. B, younger bud. C, *Capsella Bursa-pastoris* Medic., young bud ($\times 77$ circa). D, *Asparagus officinalis* L. D₁ and D₂, very young gynaeceum ($\times 47$). E, *Antirrhinum majus* L., four sections from series through a young gynaeceum ($\times 47$). F, *Aconitum Napellus* L. F₁, very young gynaeceum cut in ovary region; F₂, "south" carpel in F₁ at a higher level.

has been made by Goebel (1933, p. 1821). He describes the gynaecium as formed of megasporophylls (carpels), bearing ovules on their margins, which are incurved and fused with one another, or with those of other megasporophylls. Considering the range of variation among angiospermous gynaecia, it was a bold measure on the part of the early morphologists to seek to bring them all under this simple and uniform conception—but the event has proved that it “works”. Systematists have found it possible to use it for the description and comparison of the female organ in the plants with which they deal; Engler (1926), for instance, after more than fifty years of taxonomic work, stated definitely that he adopted the foliar carpel view for all angiosperms. The validity of the theory has, however, been disputed in recent years, and it seems necessary to present some of the evidence for it, since—as one of its opponents has pointed out—“Aucun ouvrage ne contient un exposé complet et ordonné des arguments que l’on a produits en faveur de cette interprétation” (Grégoire, 1931). An exhaustive review of the arguments for and against this theory would involve a detailed account of the structure of every type of gynaecium in the angiosperms. This is an impracticable task, but as a small step in this direction, I have made an examination of serial sections of flowers belonging to a number of both dicotyledons and monocotyledons—some of them chosen as test cases. The result has been to fill me with wonder at the insight shown by the earlier morphologists in envisaging the carpel and foliage leaf as members of the same category, when they had to study them without the advantages of modern technique. Some of the sketches in Fig. 2 show how microtome sections of young gynaecia may serve to illustrate the Candolleian theory. In Fig. 2, F_1 , the three follicles of *Aconitum Napellus* L. are drawn at a very young stage, and it will be seen that the carpellary margins bearing the rudimentary ovules are free from one another, though appressed. Freedom of carpellary margins in young monocarpellary gynaecia has been figured by McLean Thompson (1929) for a number of legumes, and by Joshi (1934, Fig. 1, 6, p. 969) for *Boerhaavia*, while it is shown here (Fig. 3, 4 a, b) for *Mirabilis*. Fig. 2, D_1 , is from a transverse series through a young gynaecium of *Asparagus officinalis* L., in which the three carpels, instead of being free like those of *Aconitum Napellus*, are united by their ovule-bearing margins. At a higher level, however, they separate (D_2), and their upper regions show the same leaf-like character as those of *Aconitum* (F_2) and *Clematis* (A_2). The similarity of the young carpel of *Ranunculus* to a leaf rudiment has been noted by Salisbury (1931, pp. 558–9). In the gynaecium of *Antirrhinum majus* L., in which each of the two carpels has its margins united, and also fused with those of the other carpel, serial sections passing upwards to the stigmatic region show that the margins of each carpel come apart near the top of the ovary (E_2), and that, above this, the two carpels separate (E_4). Moreover, it is not only shape which is explicable on the Candolleian hypothesis; internal structure, also, often follows just the lines which, on this hypothesis, might have been predicted. As an example we may take the anatomy at the base of the gynaecium in *Hypericum hirsutum* L., which is illustrated in Fig. 4, A_1 – A_3 . Here, after the bundles for the stamen phalanges have been given off, three arcs of vascular tissue are left for the

gynaeceum (A_1). Each of these arcs divides into three (A_2)—a median strand which forms a carpellary midrib, and two main laterals (marginal bundles), each of which fuses with a corresponding main lateral of an adjacent carpel. These fused laterals become the placental strands (A_3).

I have also met with certain gynaecea, showing divergences from the normal type, which, when examined by the method of serial sections, gave additional confirmatory evidence for the Candolle theory. Though there is no reason to suppose that abnormalities provide information about ancestral conditions (cf. Arber, 1931 *a*, pp. 197–200), it is an undeniable truth—indeed a truism—that aberrant forms, since they show what an organ *can* do, may sometimes throw light upon what it *is*. In an abnormal gynaeceum of *Ranunculus acris* L., for example, I have seen the ovule protruding freely from the open carpel, to one margin of which it was attached. And in peloric flowers of *Digitalis purpurea* L., in which the gynaeceum had many loculi instead of two, it was found that the *unit* which multiplied was always the carpel, in the sense of Goethe and de Candolle. Moreover, in these multicarpellary ovaries, the carpels were placed in irregular whorls round the axis, without having their own fused margins appressed to those of an opposed twin carpel, as in the normal bilocular type. The result of this freedom was that the foliar character of the margins was revealed much more distinctly than in normal foxgloves (Arber, 1932).

We may, I think, safely conclude from a study of the angiospermous gynaeceum that the Candolle theory has at least brought unity into an immense range of phenomena which otherwise would have been chaotic; even those who have least sympathy with this theory, admit its value in this respect. For instance, McLean Thompson (1934) writes that “The old carpellary view has been convenient for purposes of description. It has given a terminology which has been of service in systematics and which may yet be retained”. If, however, it has achieved this kind of validity, it has done all that a morphological theory can do; it has helped us to an “explanation” in the scientific sense—in other words, to a correlated description.

In saying that the Candolle theory fits closely to the facts which it was framed to correlate, I do not mean to imply that its application presents no difficulties. Some of these difficulties have already been discussed in a former paper where references will be found (Arber, 1933); in the two following paragraphs only so much of the argument as is necessary for our present purpose will be recalled.

In attempting to apply the Candolle theory, it is sometimes found impossible to delimit carpel from carpel, or carpel from axis; but this is only to be expected when we remember that in development the carpels are crowded together under pressure from the other parts of the flower, and that they are lateral members arising from an axis which is just dying out. Here, again, the comparison with the inflorescence helps us to understand the curious things that may happen in the flower, owing to the limitation in growth of its axis. In *Corydalis nobilis* Pers., for instance, the two uppermost flowers of a raceme may each receive supernumerary vascular strands, which, if the axis had continued its growth, would have passed on to supply its further developments (Arber, 1931 *b*, pp. 321–3, and Fig. 2,

D₁-D₃, p. 321); that is to say, these *apical flowers* show a peculiarity in their vascular relation to the *inflorescence axis*, which may throw light upon certain anomalies found in the relation of *carpels* to the *floral axis*.

Another difficulty, which has led some botanists to discard the classic interpretation, is connected with the distribution of the venation in the gynaecium. In foliage leaves the median vein is usually, though not always, stronger than any of the laterals; but, on the other hand, the placental regions of the carpel, which, on the Candolle view represent foliar margins, are often much more richly vascular than the part which is held to represent the midrib. Moreover, the secondary venation may arise from these placental veins rather than from the midrib: striking figures illustrating this point for certain follicles have been given by Hamshaw Thomas (1931). This difference in relative emphasis in the venation system is connected, by those who accept the classic view, with the ovule-bearing function of the carpel margins, and the absence of any such function in the case of the margins of the foliage leaf.

Those who do not accept the classic view have suggested other hypotheses to explain the high vascular development of the ovule-bearing regions; a common feature of these hypotheses is that they increase the number of carpels recognized in the gynaecium. Most of these interpretations need not be discussed here, since a lucid and impartial survey of them, and of the controversy which has arisen round them, will be found in a review by Bancroft (1935). One recent paper (Dickson, 1935), however—a thorough and detailed account of the gynaecium of the Papaveroideae, based upon the comparative study of serial sections—demands special consideration. For most of the Papaveroideae, and for the Cruciferae, Dickson concludes that the number of carpels must be doubled; she thus recognizes four carpels instead of two in *Glaucium* and the Crucifers. She arrives at this result by interpreting the placental regions, which are highly vascular and sometimes relatively massive, as themselves constituting a pair of “fertile contracted” carpels, while the parts of the gynaecium wall between them are taken to represent a pair of “sterile expanded” carpels. It is, indeed, undeniable that in *Glaucium*, the genus on which the account is primarily based, the placental regions are strikingly and unusually distinct, but it still appears to me much more likely that the gynaecium is truly bicarpellary, and that its peculiarities are merely due to extreme hypertrophy of the carpel margins. To postulate two types of carpel to explain such gynaecea introduces unnecessary complexity, and this becomes even more evident when we turn to the stigmatic lobes. Dickson says of *Glaucium* that “The vascular supplies to each [stigmatic] lobe actually consist of the strands of one expanded carpel *plus* half the strands of each of the fertile contracted carpels”. For other genera she gives a corresponding account: in *Macleaya*, for example, she figures the “fertile carpels” as having each a single bundle which divides into two at the top of the gynaecium. Her theory provides no explanation for the fact that the bundles of the “fertile” carpels show this tendency to bifurcate, while those of the “sterile” carpels do not show it. On the classic theory, on the other hand, according to which each stigmatic lobe receives all the strands from a single carpel—this

division is seen quite simply as *separation* of the united bundles belonging to the fused margins of adjacent carpels.

On general grounds, a serious argument against Dickson's theory is that it is an *ad hoc* hypothesis, which cannot be applied consistently even throughout the Papaveroideae. It involves explaining most of the genera in this tribe on the "sterile" and "fertile" carpel hypothesis, while retaining the classic hypothesis for *Platystigma*, *Platystemon*, and *Romneya*. The gynaecea of these three genera do not seem, however, to differ fundamentally in structure from those of the rest of the tribe; and one cannot but distrust a theory which offers a basically different explanation for the well developed fused marginal veins of *Romneya*, and for the placental veins of *Glaucium*, in which the same rich development of the marginal carpellary regions occurs, although in a more highly elaborated form.

VII. STIGMA AND TRANSMITTING TISSUE

In certain recent interpretations of the gynaeceum, there has been a tendency to treat the "stigma" as if it were a definite morphological entity. This tendency has been criticized by more than one writer (see especially Bremekamp, 1934, p. 2, footnote). As a matter of fact, the surfaces which receive the pollen merely mark the upward terminations of the tracts of tissue along which the pollen tube makes its way to the ovule; and the position of these "stigmatic" surfaces, in relation to the gynaeceum as a whole, shows a good deal of variation. Hamshaw Thomas (1934) has emphasized the incompleteness of our knowledge of what was formerly called the "conducting tissue". This, and "stylar core" which I previously used (Arber, 1934), are unsatisfactory terms for the path of the pollen tube, and the name "transmitting tissue" (suggested by T. M. Harris and adopted by Hamshaw Thomas) is certainly better; it might be well, in addition, to use "transmitting tract" for regions in which the tissue in question is merely a surface feature.

Our information about transmitting tissue goes back to Capus (1878) and Guéguen (1900-2), who showed that conduction of the pollen tube is, as a rule, effected by the inner faces of the two carpellary margins, and of the placentae which are emergences from these margins; the transmitting tissue for each carpel is thus double in origin (cf. Fig. 3, 4). The tissue involved is the epidermis, reinforced by underlying elements. A different view has been suggested by Joshi's recent work (1934). He describes the transmitting tissue (for which he uses the term "stylar canal") in various plants, and states that "the stylar canals are either continuous with the ventral traces of the carpel or make their appearance at, and occupy exactly the situation of, such traces. The conclusion is reached that the stylar canals have been derived from, and represent modified ventral bundles of, the carpels." This conclusion, if it could be substantiated, would be a very remarkable one, because a stylar canal could not perform its function unless it connected with the ovary cavity—a thing which it could scarcely do if it represented the continuation of one of the ovary bundles. In order to test Joshi's theory, I have cut serial sections of gynaecea of *Mirabilis jalapa* L., one of the species which he describes. This plant

was also dealt with formerly by Guéguen, who stated that the transmitting tissue descends vertically in the posterior wall of the ovary and expands at the bottom of the cavity into an annular mass which surrounds the funicle. My observations confirm this description (Fig. 3); they are not in accordance with Joshi's statement

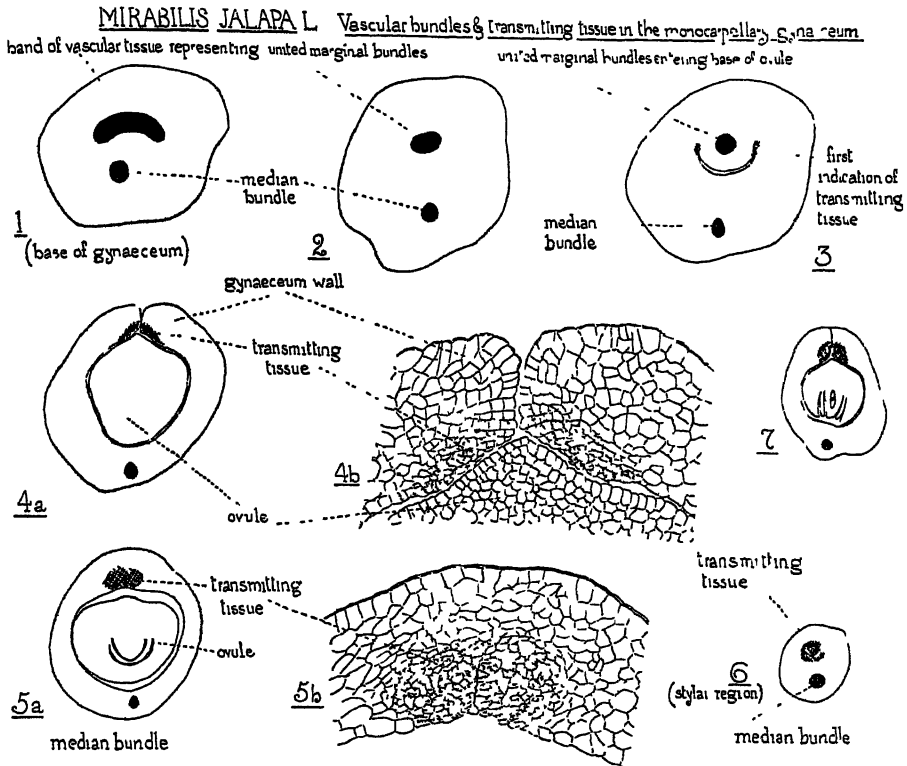


Fig. 3. *Mirabilis jalapa* L. (Cambridge Bot. Gard.). 1-6, transverse sections from microtome series from below upwards through a bud at the stage at which the style had not elongated, but the pollen-grains were already mature (at this stage carpel still open). 1, 2, 3, 4 a, 5 a, 6 ($\times 47$); 4 b and 5 b ($\times 193$ circa). 7, transverse section from a carpel of another flower to show topography of ovule, which is not seen in the series 1-6 ($\times 47$). Vascular bundles indicated in solid black, transmitting tissue cross-hatched in the low power, and dotted in the high power diagrams; this dotting is merely a conventional symbol to indicate the relatively rich, deeply staining cell contents of the transmitting tissue.

that "the styli canal occupies the place of the ventral traces of the carpel". I find that the transmitting tissue is formed as usual from epidermal and subepidermal layers, and that it appears to bear no relation to the fused ventral bundles which enter the base of the ovule (Fig. 3, 3).

There is little doubt that the description of the transmitting tissue of angiosperms given by Capus and Guéguen is, in a general way, correct in outline, though their work, having been done merely with the aid of hand sections, needs supplementing with more exhaustive microtome work. I include figures here illustrating the course of the transmitting tissue in the syncarpous gynaecium of *Hypericum*, for comparison with the monocarpellary gynaecium of *Mirabilis*. In Fig. 4, A₄,

the three ovule-bearing placenta almost meet in the centre of the ovary; their inner surfaces are clothed with a secretory epidermis which forms the transmitting

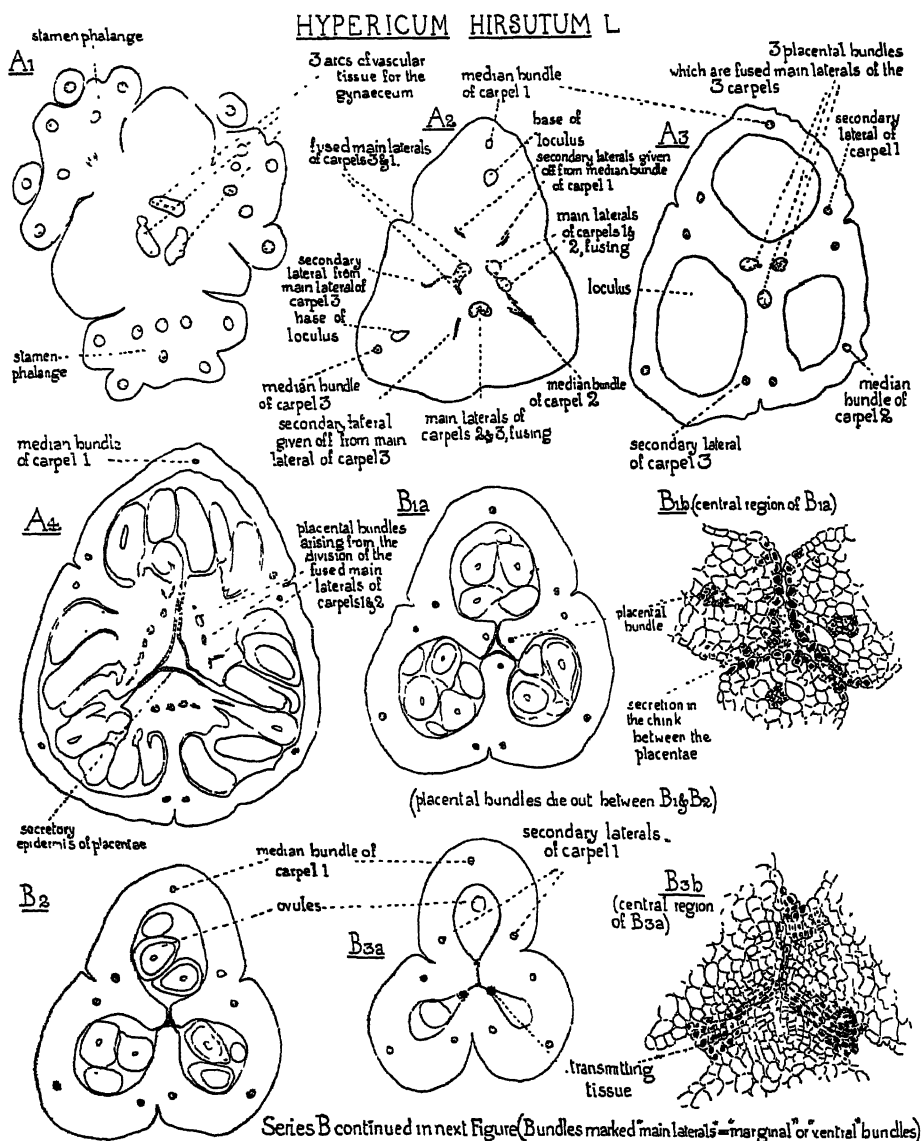


Fig. 4. *Hypericum hirsutum* L. (Wharfedale). Sections from transverse series from below upwards; A₁-A₄, through the lower part of one gynaeceum, and B₁-B₃, through the upper part of another. B₁ b and B₃ b ($\times 193$ circa); all the other figures $\times 47$. The elements of the epidermis, which forms the transmitting tract for the pollen tubes, are indicated individually in A₄, B_{1a}, and B₂.

tract. In B₁ a, another gynaeceum is shown cut at a higher level, above the insertion of the ovules. Its central region is drawn in B₁ b; the epidermis with its rich contents, and the secretion which exudes from it into the crannies, are indicated.

It will be seen from $B_1 a$ and $B_1 b$ (see also Fig. 5, C_2) that the transmitting tract extends down to a level below the tops of the placental (ventral) bundles, and that it does not replace these bundles in position, as it should on Joshi's theory. In $B_3 a, b$, the three-armed chink between the placentae has partially closed, and, at the junction between each arm and the loculus, the elements of the secretory epidermis

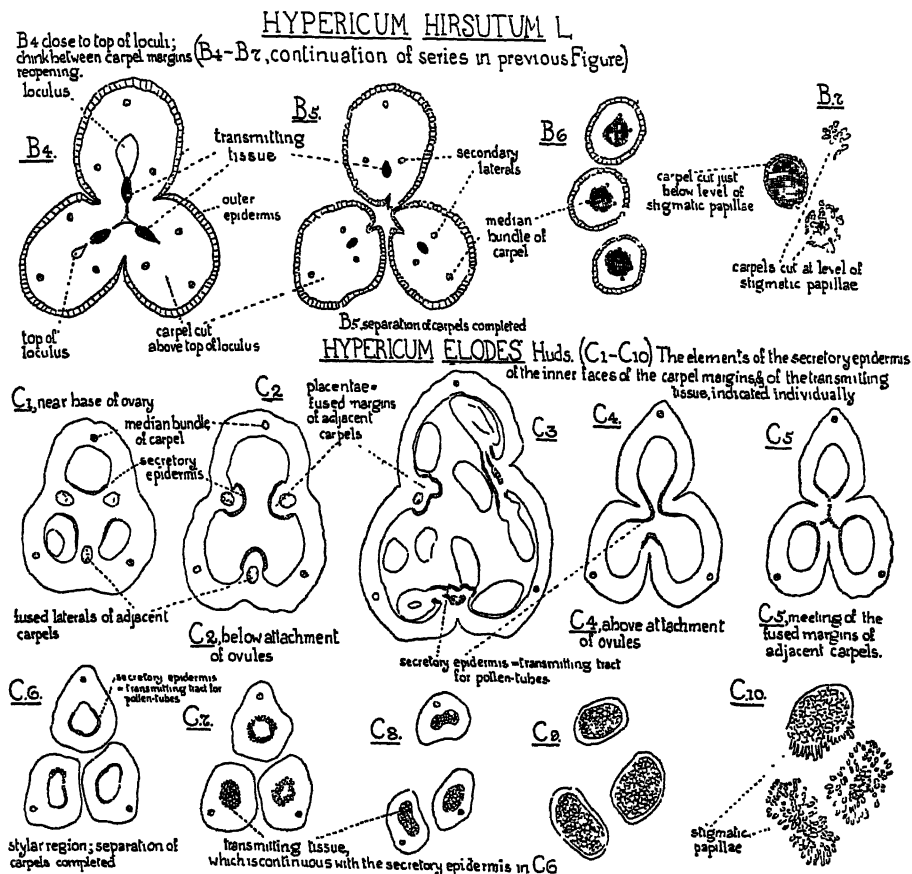


Fig. 5. B_4 - B_7 , *Hypericum hirsutum* L., continuation of series in Fig. 4, from top of loculi to stigmatic region ($\times 47$). Elements of outer epidermis indicated individually; transmitting tissue cross-hatched. C_1 - C_{10} , *Hypericum Elodes* Huds., sections from a transverse series from below upwards through a gynaecium. C_1 , near base of ovary below attachment of ovules; C_{10} , stigmas. All $\times 47$. Elements of the transmitting tract (secretory epidermis), and of the transmitting tissue into which it passes, indicated individually.

are multiplying to form the transmitting tissue. The process can be followed further in Fig. 5, B_4 , in which, owing to a slight obliquity in the section, three stages in the disappearance of the loculus and the development of the transmitting tissue can be seen. In B_5 , the styles have separated, and the transmitting tissue appears as an isolated patch embedded in the mesophyll of each carpel, but the preceding sections make it clear that it is in reality a continuation of the superficial tissue of the inner carpellary margins; it is in fact the only relic at this level of the inner (upper)

carpellary epidermis, while the whole of the outer skin of the carpel belongs to the lower epidermis, of which the elements are indicated individually in B_4 – B_6 . In passing upwards, the transmitting tissue increases in amount (B_6) until the level of the stigmatic papillae is reached (B_7). The sketches of *Hypericum Elodes* Huds. (Fig. 5, C_1 – C_{10}) show slight differences from those of *H. hirsutum*; the secretory epidermis is readily traceable almost to the base of the ovary (C_1), and open canals enter the base of the styles (C_8); but in essentials the story is the same.

We have still to consider the bearing of a study of the transmitting tissue upon the problems with which we are concerned in the present survey. This bearing is simple; since this tissue proves to be a secretory epidermis (and sometimes sub-epidermal layers) belonging to the inner faces of the carpellary margins, its existence is in no way incompatible with the Candolle view of the carpel, for localized secretory activity of the epidermis is a familiar feature of leaf structures.

VIII. GOETHE'S THEORY AND THE MEANING OF HOMOLGY

Hitherto, in discussing the flower, I have spoken in somewhat general terms about the acceptance of Goethe's theory; exactly what this acceptance implies—and, above all, what it does not imply—must now be considered. The main question is, what, precisely, is meant when we say that the parts of the flower *correspond* to, are *equivalent* to, or are *homologous* with, foliage leaves. In the first place it must be clearly understood that by "equivalence" we do not mean "identity", and that in treating the carpel, for instance, as equivalent to a foliage leaf, we are not belittling the important differences which separate these two members. This may seem to be an obvious truism, but it is necessary to stress it because, in discussions of Goethe's views, it is sometimes assumed that homologous members ought to show similarity of the closest type—almost amounting to identity—especially in the earlier stages of their history. It is curious that this idea should have arisen when one recalls that in considering *serial* homology (e.g. between such members as the hand and the foot) biologists recognize equivalence despite great differences. Failure to discriminate between homology and identity is shown by a recent critic of Goethe's views, who regards the petals, stamens and carpels as organs *sui generis* (Grégoire, 1931). He bases his conception of morphological equivalence entirely upon ontogeny; he would only regard the carpel, for instance, as homologous with the foliage leaf, if "la plante, pour produire cet organe, utilise un *primordium foliaire*". Such a condition as this seems to me to exclude the very existence of the phenomenon which he seeks to define. Before modern botanical methods were devised, no difference was detectable between the young rudiments of various organs, and it was possible to believe that they remained undifferentiated, and not committed to a particular course of development, until a relatively late stage; but as technique has been refined, the existence of distinctive organization has been revealed in earlier and earlier stages. This is demonstrated especially by the work upon the developmental history of foliage leaves and bud scales which we owe to Foster (1935 *a, b*; see also a series of earlier papers by this author and the literature which he cites). This work makes

it clear that each rudiment is committed, from an extremely early period of its life, to a path which conducts it at maturity to a determined and specific form.

Foster's results clearly preclude an ontogenetic definition for the homology of parts within the individual plant body; we have then to consider what alternative interpretations are possible. Definitions depending on phylogenetic considerations have sometimes been suggested, but our ignorance of the ancestry of the flowering plants would deprive such definitions of any permanent value. We have to remember also that the idea of homology was clearly understood by pre-Darwinian morphologists, although they attached no evolutionary significance to it. For morphological purposes we need, then, some conception of equivalence of parts which neither depends upon the facts of ontogeny, nor upon the changing visions of phylogeny, and which will bring the sound elements in pre-Darwinian views into harmony with the thought of to-day. It seems to me that these conditions are best fulfilled if we recognize "equivalence" or "correspondence" of the various members of the plant body as coming under the category of *parallelism* within the development of the individual (cf. Zimmermann, 1930, p. 227). The concept of parallelism in the evolutionary history of systematic units has played a steadily increasing role in recent years, and it would be well if the idea of a comparable parallelism between different parts of one and the same plant body could be substituted for the more rigid notions attaching to the word "homology". On these lines—treating the carpel and foliage leaf as parallel developments—we should say that the nature of the carpel can be understood by *comparing* it to an infolded foliage leaf bearing ovules on its marginal regions; this is a very different thing from saying, in a literal sense, that the carpel is an infolded foliage leaf. When we turn to Goethe's original essay, we find that it is possible to do justice to his opinions in terms of parallelism, although he gave expression to them himself on different lines, since he treated all the appendages both of the floral and vegetative axes as protean forms of one hypothetical *type appendage*. He explains that he adopted the word "leaf" (Blatt) for this fundamental organ, because he had to give it some name, but it is evident that the term did not satisfy him. It is regrettable that his dissatisfaction did not go far enough to make him discard the vernacular word "Blatt" altogether, since it inevitably suggests foliage. The term "phyllome", for this purely abstract type appendage, was coined at a later date by Nägeli (1884); if it had been used from the first, it might have saved subsequent writers from much confusion of thought—a confusion not completely avoided even by Goethe himself. Unfortunately for science, the artist in Goethe was stronger than the philosopher, and he was liable to slip into a kind of picture thinking, which deceived him into attributing an objective reality to concepts which were, in fact, abstractions. He seems never to have grasped the implications of a remark on the subject made to him by Schiller on the historic occasion when they discussed Goethe's botanical ideas. He tells us that he demonstrated his theory of the metamorphosis of plants to Schiller in vivid fashion, and that with many characteristic strokes of the pen he caused a symbolical plant to arise before his eyes. When he had finished, Schiller shook his head and said, "This is not an experience (*keine Erfahrung*); it

is an idea"; to which Goethe replied, "I am very glad to find that I have ideas without knowing it, and that I even see them with my eyes" (*Glückliches Ereigniss*, pp. 13-20, in Goethe, 1893). If Goethe had realized the full force of Schiller's criticism, and had modified his own outlook so as to meet it, botanical morphology might have been placed from the first upon a firmer basis.

IX. GOETHE'S THEORY AND PHYLOGENETICS

Though Goethe sometimes lost sight of the abstract quality of his concepts, he was at least fully aware of it when he insisted on relating the phenomena of metamorphosis to one another *in both directions*. He regarded the flower as a contracted vegetative shoot, but he also regarded the vegetative shoot as an expanded flower; and he held that it was as legitimate to call a foliage leaf an expanded sepal, as to call a sepal a contracted foliage leaf. Unluckily the significance of this reversibility was lost upon his successors, who failed to realize that relations of this kind, which can be represented with equal truth in either direction—backwards or forwards—have nothing to do with derivations, and can play no part in evolution, which is essentially an irreversible process. The fact that the concepts of pure morphology have been dragged into evolutionary discussions, to which they are, in their nature, alien, is one of the symptoms of the nineteenth-century tendency to the restatement of scientific problems in terms of history (Collingwood, 1924, pp. 53, 187). Darwin's work, in particular, was held to have set biology once and for all upon an historical basis, and morphology was defined as "the study of structure in the light of the theory of descent". To many workers this diversion of biology into historical channels was a welcome relief, since it transformed theoretical botany into something material and tangible, amenable to picture thinking, and not demanding mental activity of a metaphysical type. In the eager rush to transmute the study of plants into history, a crude version of Goethe's ideas was lifted bodily from its context and forced into an evolutionary frame. Darwin's own share in this confusion may be illustrated by the following passage from the first edition of *The Origin of Species*—a passage which is repeated without essential alteration in the last edition which he revised (the italics are mine): "Naturalists frequently speak of...the stamens and pistils of flowers as metamorphosed leaves;...but it would...probably...be more correct...to speak of [stamens and pistils on the one hand, and leaves on the other] as having been metamorphosed, not one from the other, but from some common element. *Naturalists, however, use such language only in a metaphorical sense...On my view these terms may be used literally.*" This identification of an abstract (or, as Darwin calls it, a "metaphorical") concept, with an objective historical reality, has been accepted with curiously little criticism, and even in recent literature we may find an explicit statement that the idealistic morphology of Goethe supplies something which can often be taken over directly into phylogenetics (Zimmermann, 1930). Goethe's theory was, however, in no sense intended to serve as an account of the past. He saw the life of the individual plant as a drama in time, but only within the limits of its own life cycle; his mind pre-

served the unities, and his view was definitely non-historical. On purely morphological lines, and with the aid of such facts as were generally accessible in his day, he brought forward reasons for the probable truth of his theory, and the more technical evidence, that has since become available, is of the same strictly morphological type. Those who visualize the flower and its parts as having been derived phylogenetically from a vegetative shoot with foliage leaves, and who suppose that the carpel has at some stage gone through an actual process of infolding, are replacing a *morphological* idea, valid within its own sphere, by an *historical* picture, which belongs to a different world of thought, and for the validity of which the morphological concept provides no evidence. Morphological and phylogenetic concepts belong to different categories, and only confusion can come of the attempt to reduce these categories to one.

X. POST-DARWINIAN PHYLOGENETICS

We must now consider certain interpretations of the flower which belong to the phase of phylogenetic morphology whose onset has been indicated in the preceding paragraph. The most recent of these may be taken first, since it has a direct bearing upon the question whether the classic theory of the carpel is to be retained. Since on Goethe's view the carpel throughout the angiosperms is open to description in terms of the leaf, it becomes probable that any ancestral stocks from which the angiosperms were derived should be assumed to have shared this character. This fact has led Hamshaw Thomas to the rejection of Goethe's view, since he considers that he has found, in certain fossil plants of Mesozoic age, indications of what the ancestral angiosperm may have been like, and these indications are not consistent with Goethe's morphological ideas. Hamshaw Thomas's views are mainly based upon a consideration of the female reproductive organs of the Caytoniales—a group which he has made peculiarly his own by his well-known and remarkable studies. The fructification of *Caytonia* or *Gristhorpia* (Thomas, Hamshaw, 1925 and later papers) consists of a slender "axis", apparently dorsiventral (described at different times as "foliar" or as a "stem"), bearing stalked seed-containing bodies in opposite pairs. Each of these bodies recalls an anatropous ovule in shape, and has a stigmatic region at the base near the stalk. On the strength of a comparison with this fructification, Hamshaw Thomas proposes a new interpretation of the angiospermous carpel (1931, 1932, 1934, 1935); but after a study of his descriptions and figures, I find it difficult to believe that the resemblances between these two structures are of such a nature as to justify the comparison. The difference between them is sufficiently indicated by the extreme complexity of the scheme which Hamshaw Thomas finds it necessary to introduce in order to relate the carpel of the angiosperm to the caytonialean fruit body. He proposes to regard the carpel as originally composed of four or five distinct parts—two fertile branch tips forming the placentae, and two concrescent cupules forming the ovary wall, with sometimes, in addition, the relics of a stem between the cupules. If this is to be the interpretation of a single carpel, a multicarpellary gynaecium must indeed represent a branch system of excessive complexity. Those who are concerned with the

modern flower may well feel that it would require evidence of a more cogent character than has hitherto been produced to convince them that they ought to discard the successful simplicity of the Candolleian carpel in favour of this elaborate concept.

It is not only students of the living flower who find Hamshaw Thomas's theory difficult to accept; it is also open to criticism from the palaeobotanical standpoint. Hirmer (1935), for instance, regards the pteridosperms (with the Caytoniales, which he considers to be a Mesozoic offshoot from this group) and the angiosperms as two series which are convergent as regards macrophyllly, but in other respects are so divergent that they cannot be included in one phylum. He thinks that the attempts at a closed type of gynaecium found among the pteridosperms have no more relevance to true angiospermy than the attempt at a seed, found in *Lepidocarpon*, bears to the seed habit of the higher plants.

In his comparison of the angiospermous flower with the caytonian fruit body, one cannot but feel that Hamshaw Thomas lays undue stress upon a single character—the closure of the gynaecium. Among the many features which separate the true flowering plants from other groups, that of “angiospermy” stands in no predominant position; but a fictitious importance is sometimes ascribed to this character, simply because it happens to have been recorded in the name of the class. One character cannot carry us very far in discussions of affinity. Indeed, as Harris wrote concerning the ancestry of the angiosperms (1935), “no two classes can be regarded as related until *all* the organs of the one can be interpreted simply in terms of those of the other”.

Another view of the history of the flower, which was suggested at a much earlier date than the caytonian theory, depends upon a comparison on broad lines between the reproductive shoot of a second Mesozoic group—the Bennettitales—and the flower of the angiosperms (Arber, E. A. N. & Parkin, 1907). In its inception, this theory offers a further contrast to that of Hamshaw Thomas, for while his ideas about the angiosperms were primarily the outcome of palaeobotanical work upon plants of earlier date, Newell Arber and John Parkin started from the other direction, and deduced the characters of the hypothetical primaevial flower from a comparative study of the living angiosperms themselves. The appearance of the first volume of Wieland's work upon the American Bennettitales (1906) merely revealed to these authors the actual existence of a type of reproductive shoot from the Mesozoic rocks which complied with many of the conditions which they had already formulated. The chief difficulty of the comparison between the angiosperms and the Bennettitales lies in the organization of the female reproductive organ. I am inclined to think that this difficulty is insuperable, although as Sahni has recently pointed out (1935)—“as speculations go, the direct closing in of the interseminal scales round the ovules of a cycadeoid so as to form closed carpels does not seem more far-fetched than the derivation of a *Caltha* carpel from a structure of the *Caytonia* type”. In other respects there is certainly more to be said for the comparison of the angiosperms with the Bennettitales than with the Caytoniales—a group which “does not foreshadow any known primitive angiosperms, as the

Bennettitales or even the Gnetales seem to foreshadow the Magnoliales, at least in floral organization, wood anatomy and other features" (Sahni, 1935). Nevertheless, despite the acknowledged resemblances between the angiosperms and the Bennettitales, it seems most reasonable to regard them as groups which are independent, though showing various parallels. We may recall that Hagerup (1934, 1936) has recently emphasized the resemblances between the gymnosperms (*Juniperus*), the Gnetales and certain angiosperms, from which he draws the conclusion that these groups form one continuous phylum; but here again it can scarcely be doubted that the similarities, which he assumes to be due to descent relationship, are merely instances of those parallelisms which are so common between all the groups of plants which have reached the seed-habit stage. Harris (1935) probably comes nearest to the truth when he writes that "The various classes of seed plants can at best only provide useful ideas by exhibiting types of workable mechanism which have in fact been evolved and which therefore may suggest features which might have existed in ancestral Angiosperms."

The validity of the caytonialean and bennettitalean theories of the angiospermous flower cannot be tested merely by a structural comparison of the groups whose affinities are in question; for both these theories are bound up also with the *a priori* supposition that the clue to the origin of the flowering plants may be buried in the Mesozoic rocks. It is true that, thirty years ago when Newell Arber and John Parkin wrote "The origin of angiosperms", this supposition seemed well based, but with the passage of time its probability has waned. The geological record has not produced the long-expected synthetic types connecting the main groups: "Always the common ancestor eludes us and retreats further and further into the past" (Bather, 1931). The degree to which phyletic lines, both in animals and plants, have been traced back and back in time, has awakened biologists to the importance of parallelism in evolution, and has suggested to some minds that the search for ancestral connecting links is of the same order of hopefulness as the search for the philosopher's stone. Even those whose pessimism is less extreme, may well feel to-day that it is useless to examine Mesozoic strata for indications of the primeval structure of angiosperms—a group which shows every sign of maturity when it comes to our knowledge in the Cretaceous (cf. Arber, 1928 *b*, p. 71). The high differentiation of the group in its (to us) earliest appearance is one of the puzzles of palaeobotanical history; but it finds close parallels in the history of mammals and of certain cryptogams. One is tempted to wonder, also, whether there is any relation between these puzzles and similar problems in so remote a field as the history of art. It has been stated that, "astonishing as it may seem, no Chinese bronze has yet been discovered which one could label with certainty as 'primitive'. The story apparently begins with examples showing such technical skill and elaboration of design as to postulate a long antecedent period of evolution and development" (Koop, 1935).

Such enigmas as the sudden appearance of the angiosperms, and its various parallels, may possibly be taken as suggesting that there is something radically wrong in our general view of the evolutionary process, and that a crucial alteration

in focus is needed before we can hope for clear illumination upon such problems as the origin of the flower.

Another attempt at a phylogenetic interpretation of floral morphology is that of Zimmermann (1930). The lines upon which this attempt is made differ markedly from those followed by Arber & Parkin, or by Hamshaw Thomas, since Zimmermann chooses a very much earlier starting point. He bases his whole interpretation on the idea that the type of structure shown by the Devonian Rhyniaceae is primordial for the Cormophytes. This procedure is open to the fundamental objection that we are by no means certain that *Rhynia* was a primitive type. As Goebel wrote (1933): "Möglich, dass sie eine sehr primitive, möglich auch, dass sie eine stark abgeleitete war!" If, however, we put this objection on one side, and follow Zimmermann's argument as he passes the vegetable kingdom in review, we find that *Rhynia* certainly serves to some extent as a useful term of comparison so long as he is dealing with the vascular cryptogams, but that when the higher plants are reached the clue seems to be lost. The part of the book dealing with the angiosperms is relatively confused and incoherent—an inevitable result of the attempt to fit the facts of floral structure into a scheme based upon the organization of a group with which it may well be that the forbears of the angiosperms never had any connexion.

XI. THE REACTION FROM PHYLOGENETICS

In recent years there has been a marked reaction among students of the flower against the identification of morphology with phylogenetics. Zimmermann (1930, 1934), recognizing the inherent difficulties in the way of race phylogeny ("Sippenphylogenie"), makes an interesting effort to replace it to some extent by "Merkmalsphylogenie", i.e. the phylogeny of single characters; but this, when analysed, seems to be little more than a version of idealistic morphology, with the attempted addition of a time element. The table (1930, pp. 334-7) in which he arranges numerous floral and vegetative characters of the angiosperms as "primitive" or "derived", shows that, though "Merkmalsphylogenie" claims to be independent of "Sippenphylogenie", it continually slips back into it.

The reaction against phylogeny is merely foreshadowed in Zimmermann; but it is fully developed in the work of McLean Thompson (1934*a* and other papers of this series; 1934*b*, 1935; for critical discussion see Clapham, 1934 and Bancroft, 1935, pp. 90-4). McLean Thompson's standpoint is so remote from that of phylogenetics as to lead him to the conclusion that "the real problem of floral morphology is that of the physiology of growth of a sporogenous axis". At present, however, we are so far from a synthesis of morphology and physiology that morphological problems cannot be restated in terms of physiology, except by a process of abstraction which omits essential elements. For this reason I think that, despite the interest of the ontogenetic data which he provides, McLean Thompson's *theories* hardly come within the province of a morphological discussion. From the point of view of theoretical morphology, the great value of his contribution seems to me to lie in his emphasis on holism, as in his dictum—"the mystery of the carpel is that of the flower as a whole".

XII. "GESTALT" MORPHOLOGY

Another recent writer who discards the phylogenetic aim—Wilhelm Troll—differs fundamentally from McLean Thompson, since he contends that morphology can never pass over into physiology. He returns to the standpoint of Goethe in holding that morphology *is* comparative morphology. He considers that the subject was developing in a more hopeful direction in the period before it was diverted by Darwin's influence into the service of evolutionary speculation—a view which had already been suggested elsewhere (Arber, 1925). It will be worth while here briefly to indicate the scope of Troll's book on the flower (1928), since little attention has been paid to his ideas in this country, despite an interesting review by Rendle (1929). This neglect is not unnatural, since Troll's notions do not lend themselves to concise expression, and it takes time to disengage them from the somewhat confusing background upon which he presents them.

Troll considers that, according to the point of view from which the flower is regarded, it may be treated as (i) a biological type, (ii) an organization type, or (iii) a "Gestalt" type. When regarded as a *biological type*, the flower is seen ecologically, in relation to insect visitors and other environmental factors. When regarded as an *organization type*, it is understood in a sense which would ordinarily be called morphological; that is to say, it is treated structurally, as formed of units such as petals, carpels, etc., arranged according to a definite architectural scheme. The flower as seen from these two familiar standpoints is not, however, the subject with which Troll is concerned; his book deals with the flower from the third standpoint—as a "*Gestalt*" type. In trying to convey the gist of his "*Gestaltlehre*", one is faced by the serious difficulty that his views involve a reorientation of the orthodox outlook, and that the English language and mode of thought are ill adapted for expressing the sort of idea to which he desires to give currency. A large part of the book is devoted to an extremely detailed analysis of various resemblances which have no phylogenetic basis, such as those between pseudanthia (inflorescences simulating flowers) and euanthia (simple flowers). Most botanists dismiss resemblances of this kind as empty curiosities which are scarcely worth thinking about, or else label them as "adaptations", but Troll finds in them the text for a new morphology. His intensive study of these resemblances—which have long been recognized in outline—gives a cumulative impression, to which it is impossible to do justice in a brief summary. I will now only attempt to make his meaning emerge from a few examples; his own descriptions and figures must be consulted before his conclusions can be evaluated fairly.

The similarity of the involucre to a calyx, and of the ray florets to a corolla is especially noticeable in some composites in which the number of bracts and ray florets is small. *Chrysogonum virginianum* L., for instance, has an involucre consisting of five bracts in one whorl, recalling a calyx, and five ray florets suggesting petals—the whole producing a strikingly flower-like effect. As Troll's emphasis is on form rather than on organization, he is led to pay special heed to petals, since they are primarily responsible for the form of flowers; this is a contrast to the usual

view, in which the corolla is treated as of little import in comparison with the "essential organs". By a statistical analysis of the colour groups to which the petals of the Choripetalae, the petals of the Sympetalae, and the ray florets of the Compositae can be assigned, he shows that the pseudopetals of the Compositae agree in this respect with the petals of the Choripetalae, rather than with those of the other members of their own grade, the Sympetalae. Moreover, this is not true only of the ground colour of the ray florets, but it extends to the form and pattern of their colour markings (Zeichnungen). Troll shows that the "brush work", as perhaps it might be called, on the rays of the composite capitulum, belongs rather to the type of that found on the petals of the Choripetalae than to that on the lower lips of dorsiventral sympetalous flowers—the category to which these rays actually belong from the "organization" standpoint. The ray florets are not the only material from which a pseudocorolla may be constructed. In *Helipterum Manglesii* F. Muell., for instance, the yellow disc florets are surrounded by petaloid bracts giving much the effect of ray florets. So far we have been thinking only of an inflorescence simulating a flower; but the complexity may go further, and an *inflorescence of inflorescences* may adopt the form of a simple flower. *Syncephalanthia decipiens* Bartl. has a reproductive shoot of this type. There is a terminal capitulum around which generally four lateral capitula are grouped, standing almost at the same height. The centre capitulum possesses the disc type of floret alone. The lateral capitula, on the other hand, though consisting partly of disc florets, each have also one or two ray florets on the side away from the central capitulum; the result is deceptively like a simple capitulum with disc and ray florets. Since Troll's book was published, a comparable example has been described from among the Gramineae, in which an *inflorescence of inflorescences* simulates a simple inflorescence. In the bamboo genus *Schizostachyum*, McClure (1934) has shown that there are "pseudospikelets" which on analysis are found to consist of a series of true spikelets aggregated into one apparent spikelet. In addition to the composites, Troll has studied the remarkable pseudanthia produced by the genus *Euphorbia*. He shows that they resemble single flowers even in unexpected details. He compares, for instance, the involucre of *Euphorbia fulgens* Karw. with the corolla of *Nerium Oleander* L.; they are alike in having a basal tube with free distal lobes, in the presence of appendages at the mouth of the tube, and in the contorted aestivation of the free lobes. Other similarities with which Troll deals are those between *Aristolochia* and certain aroids, the Polygalaceae and Papilionatae, etc. Moreover, he goes further and discusses the resemblance which the flower-like grouping of parts, sometimes found *within* a single flower, bears to a euanthium. In *Iris* he distinguishes each of the three perianth members of the outer cycle, with its associated stamen and styler branch, as forming a "secondary flower".

The upshot of Troll's survey of the flower is that he deduces the existence of a flower "Gestalttypus" (form type)—an abstract concept which may find its manifestation in part of a single flower (secondary flower); a single flower (euanthium); an inflorescence (pseudanthium); or an inflorescence of inflorescences (pseudcephalanthium).

In expressing his ideas Troll is hampered by the difficulty (which he recognizes) of finding satisfactory terms—a difficulty akin to that which Goethe experienced in finding a name for his type appendage. It is clear that the words “pseudanthium” and “euanthium” convey a misleading impression, since, on Troll’s view, the pseudanthium does not *simulate* the euanthium; both, on the contrary, are different manifestations of a single abstract type form, and one is no more “pseudo” than the other. Troll’s concept of the Gestalttypus of the flower is, indeed, itself open to the same practical objection that besets Goethe’s concept of the type appendage—the objection that it is hard to find words in which to discuss such abstract ideas. It was suggested earlier in this study (p. 173) that the use of the *type appendage* concept might be avoided by substituting the idea of *parallelism of organization* within the plant body. Similarly the Gestalttypus concept may be replaced by the idea of *parallelism of form* or *configuration* between the reproductive shoots of different flowering plants, a parallelism which may recur in parts of shoots, individual shoots, or shoot complexes. Even if this suggestion is accepted, we are still faced by the difficulty that neither “form” nor “configuration” is adequate as a translation of “Gestalt”; it seems, indeed, best to retain the German word, which has already found its way into our language in the expression “Gestalt psychology”. An analogy—though a remote one—may perhaps make the significance of “Gestalt” clearer. Suppose we imagine a group of art students with a model posed before them; and suppose that one of them represents the figure in a black and white line drawing, one in water colour, one in pastel, one in carved wood, and so on. The results might be said to belong to the same Gestalt type, since they all represent the same model, but the representation is “organized” in a different medium in each case.

As Troll points out, the idea of the Gestalt type was foreshadowed by Delpino (1868–70) in his work upon pollination mechanisms. The chief difference between his outlook and that of Troll, is that Delpino was essentially a teleologist, whereas Troll rejects adaptational explanations. He shows that the resemblances with which he deals are often carried into minute details to which no utility can reasonably be attributed. Allowing for this difference, Delpino’s suggested “idea tipica”, or “concetto biologico”—which remains one, though founded on divergent schemes of organization—may be identified with the Gestalttypus. His remark that “Il concetto biologico supera e travolge il concetto morfologico”, might be translated into Troll’s terms as, “The Gestalt type overrides the organization type”. Delpino’s view is perhaps best summarized in the sentence, “Nella costituzione degli esseri viventi la forma è l’ elemento transitorio, l’ idea tipica è l’ elemento costante” (In the constitution of living things, the organization is the transitory element, the Gestalt type is the permanent element). Delpino, after comparing the flowers of the Papilionatae and Polygalaceae, concluded that these resemblances indicate the existence in nature of a law of which they are the result and the expression. The work both of Delpino and of Troll points, indeed, to the existence of some principle lying at the back of the Gestalt type of the flower, which we have yet to discover. I think that the great merit of the “Gestaltlehre” lies in its emphasis on the fact that certain familiar phenomena—which botanists tend to regard blankly, or to

dismiss with some teleological catchword—must be in reality full of significance. If the key to this significance could be found, the interpretation of the flower might enter upon a new and more hopeful phase.

XIII. SUMMARY

In the introduction to this study, the chief phases in the interpretation of the flower, from Goethe's day onwards, are briefly indicated in their historical sequence. Goethe's theory of the equivalence of the vegetative shoot to the flower, in the angiosperms, is then discussed and an attempt is made to evaluate the evidence for it. It is shown that this theory, if understood in a broad sense, harmonizes with the modern holistic trend in morphology. It is suggested that the flower is comparable with a vegetative shoot in a condition of permanent infantilism. Special emphasis is laid upon the inflorescence as offering, in some respects, an intermediate term between the vegetative shoot and the flower.

After a brief consideration of bracts, sepals, petals and stamens, the Candolle theory of the carpel is discussed, and it is concluded that it has been peculiarly successful in providing a framework for the vast plexus of facts which it is its task to correlate. Some of the difficulties which have been felt in regard to this theory are considered, with special reference to recent work on the gynaeceum structure of the Papaveroideae. The stigma and "transmitting tissue" are then discussed, and it is concluded that there is nothing in the behaviour of this tissue which is out of harmony with the Candolle theory of the carpel.

An attempt is made to arrive at a more precise notion of the meaning to be attached to *correspondence*, *equivalence* and *homology*, when these terms are used in connexion with Goethe's comparison of the vegetative and reproductive parts. It is suggested that these terms are best translated into the language of modern thought by the word *parallelism*, thus avoiding the use of Goethe's type concept, which cannot be safely employed unless its abstractness is constantly borne in mind.

The nineteenth-century phase, in which morphological ideas were lifted bodily into an historical setting, is then discussed, and emphasis is laid upon the danger of thus confusing two irreducible worlds of thought. Certain attempts which have been made to relate the flower of the angiosperm to the reproductive organs of plants of earlier geological periods are briefly criticized.

In the concluding sections, attention is drawn to the current reaction against phylogenetic morphology, and in favour of the purely comparative morphology contemplated by Goethe. A slight sketch is given of Delpino and Troll's theories of the flower, in which "form" is considered as distinct from "organization". Whether these views are accepted or not, the "Gestaltlehre" is at least an indication that the morphological ideas, which Goethe initiated before the end of the eighteenth century, may even to-day suggest fresh approaches to the problem of the interpretation of the flower.

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ADDENDUM. 23 November 1936

Since the present review was in print, the memoir to which Newman, I. V. (1936) forms the preliminary note, has appeared in *Proc. Linn. Soc. N.S.W.* **61**, 56-88. The conclusion is reached (p. 75) that the legume in the two species of *Acacia* examined "is a lateral laminar organ developed on an apex which is immediately suppressed; that the initiation and differentiation of the legume is not significantly different from those of a vegetative leaf; and that the ovules are formed on the margins of the lamina of the legume".

I have spoken in Section II of the nature of the free-central placenta in the Primulaceae as still a matter of controversy, but the subject has since been put upon a different plane by Dickson, J. (1936), "Studies in floral anatomy III. An interpretation of the gynaeceum in the Primulaceae", *Amer. J. Bot.* **23**, 385-93. Dickson's work fully establishes her conclusion that "the 'free-central placenta' of the Primulaceae may be interpreted as the fused margins of five carpels (\pm residual axial tissue)".

A summary of Foster's work, to which reference is made in Section VIII, has now appeared (1936), "Leaf differentiation in angiosperms", *Bot. Rev.* **2**, 349-72.

The following titles should be added to the references:

- Grégoire, V. (1935). "Sporophylles et organes floraux, tige et axe floral." *Rec. Trav. bot. néerland.* **32**, 453-66.
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ÜBER DRÜSEN-NERVENZELLEN UND NEURO- SEKRETORISCHE ORGANE BEI WIRBELLOSEN UND WIRBELTIEREN

VON ERNST SCHARRER UND BERTA SCHARRER
(Neurologisches Institut der Universität Frankfurt a. M.)

(Received 29 February, 1936)

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I. EINLEITUNG

IN den letzten Jahren wurde eine Reihe von Befunden erhoben, aus denen hervorgeht, dass bei Wirbellosen und Wirbeltieren gewisse Nervenzellgruppen sekretorisch tätig sind und dass ihre Elemente in ihrem feineren Bau das Bild von Drüsenzellen bieten. So berichteten Dahlgren (1914) und Speidel (1919, 1922) über grosse auffällige Zellen im terminalen Rückenmarksabschnitt von Rochen und Knochenfischen, die kolloidartige Tropfen produzieren. Diese Beobachtung fand aber nicht das ihr gebührende Interesse. Unabhängig von Speidel konnte dann E. Scharrer (1928) im Zwischenhirn der Elritze (*Phoxinus laevis*) eine lebhaft sekretorische Tätigkeit der grossen Ganglienzellen des Nucleus praecticus feststellen. Dieser Befund schien zunächst ebenso wie der von Dahlgren und Speidel vereinzelt dazustehen, bis es gelang in dem nordamerikanischen Fisch *Fundulus heteroclitus* (1930) einen weiteren Vertreter der Knochenfische ausfindig zu machen, der besonders schöne Bilder neurosekretorischer Vorgänge bot, wodurch eine allgemeine Untersuchung der Fische veranlasst wurde (1932 a). Dabei konnten die gleichen Erscheinungen an der gleichen Stelle des Gehirns bei einer Anzahl von Arten des Meeres bzw. des Süsswassers gefunden werden, während bei anderen wieder entsprechende Bilder vermisst wurden. Die Ausdehnung der Untersuchungen auf Amphibien (1933 d), Reptilien und Säuger (1933 a, b, c) ermöglichte weiterhin eine neue Erklärung der eigenartigen histologischen Bilder in den homologen Zwischenhirnkernen des Menschen. Beim Menschen hatte Poppi schon 1930 aus der Beobachtung siderophiler Granulationen und reichlicher Zellipoide auf eine innersekretorische Funktion der Hypothalamuskern geschlossen. In der Tat konnte in einer Reihe von neueren Untersuchungen (Scharrer & Gaupp, 1933; Gaupp, 1934, 1935; Gaupp & Scharrer, 1935; Divry, 1934; Roussy & Mosinger, 1934; Peters, 1935) gezeigt werden, dass beim Menschen grundsätzlich die gleichen Verhältnisse vorliegen wie bei den Tieren. Die "Zwischenhirndrüse" kommt also offenbar allen Wirbeltieren zu und "Drüsen-Nervenzellen" finden sich wenigstens bei den Fischen ausser im Zwischenhirn auch in anderen Bezirken des Zentralnervensystems. Unabhängig von diesen Befunden an Wirbeltieren beschrieb Hanström (1931, 1933, 1934 a, b, c) bei verschiedenen Crustaceen drüsig funktionierende Zellgruppen innerhalb des Zentralnervensystems und verglich sie mit unseren Beobachtungen bei Wirbeltieren. Leider gab Hanström in diesen Mitteilungen über das sogen. "Organ X" der Crustaceen keine deutliche Abbildung der betreffenden Zellen, so dass es nicht möglich war Übereinstimmung und Verschiedenheit im Vergleich zu den Drüsen-Nervenzellen der Wirbeltiere festzustellen. Solche Abbildungen gab B. Scharrer (1935) von opisthobranchiaten Schnecken und bewies hier die Übereinstimmung der Verhältnisse bei den Wirbeltieren und den Wirbellosen. Die weiteren Beobachtungen von Weyer (1935) an Bienen und von B. Scharrer (1936) an Würmern zeigten auch für die Wirbellosen das allgemeine Vorkommen sekretorisch tätiger Nervenzellen. Die bisherigen Untersuchungen erstrecken sich also mit vergleichbaren Resultaten von den Würmern bis zum Menschen.

Da es sich um eine grundsätzlich wichtige Frage handelt, ob so hoch spezialisierte Elemente wie die Nervenzellen im Stande sind neben ihrer nervösen Funktion auch eine sekretorische zu übernehmen bzw. ob sich Nervenzellen innerhalb des Zentralnervensystems zu Drüsenzellen umwandeln können, erscheint es nützlich den derzeitigen Stand dieser Frage darzustellen. Der Zeitpunkt für eine solche Zusammenfassung ist auch insofern günstig, als die *histologischen* Feststellungen soweit fortgeschritten sind, dass ein vorläufiger Abschluss berechtigt ist. Für die weitere Untersuchung müssen *experimentelle* Methoden herangezogen werden um die physiologische Bedeutung der neurosekretorischen Vorgänge aufzuklären. Die vorliegende Arbeit würde nach unserer Meinung ihren Zweck dann erfüllt haben, wenn sie zur Nachprüfung unserer Befunde anregen würde und damit zu deren Erweiterung und Ergänzung. Bemerkt sei hier auch, dass im Folgenden nicht nur die Ergebnisse unserer eigenen, bereits publizierten Untersuchungen zusammengefasst und die Mitteilungen der Autoren, die über ähnliche Beobachtungen berichteten, zitiert werden, sondern dass auch eine Reihe von noch nicht anderwärts publizierten Resultaten hier erstmals mitgeteilt werden.

Das Material erhielten wir, soweit es sich nicht um einheimische Tiere handelt, zum kleineren Teil von der Staatlichen Biologischen Anstalt in Helgoland, zum grösseren Teil konnten wir es in mehrmonatlichen Aufenthalten an der Zoologischen Station in Neapel (Herbst, 1928, 1934) fixieren. Schliesslich hatten wir Gelegenheit Wirbellose und Wirbeltiere während einer dreimonatlichen Afrikareise im Sommer 1935 für histologische Untersuchungen zu sammeln.

II. WIRBELLOSE

(1) ANNELIDA

Die am tiefsten stehende Tiergruppe, bei der bisher sekretorisch tätige Zellen im Zentralnervensystem festgestellt werden konnten, sind die Würmer. Es wurden elf Arten untersucht. Einwandfrei positiv war der Befund bei einem Vertreter der marinen Polychaeten, *Nereis virens* Sars. Im Gehirn von *Nereis* finden sich kaudal und dorsal zwei symmetrisch liegende, ziemlich ausgedehnte Gebiete von deutlich drüsenartigem Charakter. In diesen Bezirken sind drei verschiedene Typen sezernierender Nervenzellen zu beobachten:

(1) Spindelförmige Zellen in der Umgebung der hinteren Augennerven enthalten in ihrem maschenförmigen Plasma kleinere und grössere Tröpfchen eingelagert. Diese färben sich mit Säurefuchsin kräftig rot ebenso wie die später zu beschreibenden Zelleinlagerungen bei höheren Tieren.

(2) Rundliche Zellen fallen gegenüber den gewöhnlichen Ganglienzellen von *Nereis* durch ihr stark vakuolisirtes Plasma auf. In diesen intrazellulären Vakuolen kann eine äusserst fein granulirte Substanz beobachtet werden.

(3) Besonders auffällig in dem hier beschriebenen Gehirnbezirk ist eine grosse Anzahl rundlich-blasiger Gebilde. Es handelt sich hierbei offenbar um Reste ehemaliger Ganglienzellen, die sich wohl bei der Bildung von kolloidartigen Sekretstoffen erschöpft haben. Der Kern ist nämlich, wenn er nicht überhaupt zu

fehlen scheint, an die Wand gedrückt. Ebenso ist vom Plasma nur noch eine sehr dünne Randschicht vorhanden. Den ganzen Raum solcher ehemaliger Ganglienzellen beanspruchen eine oder mehrere grosse Vakuolen.

Sie enthalten in der Regel grossere, mehr oder weniger regelmässig runde

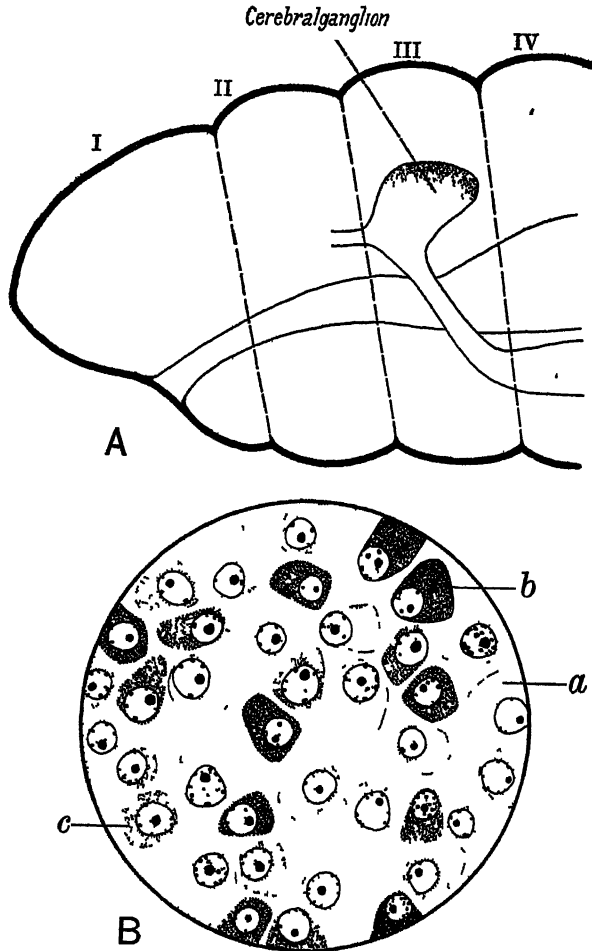


Fig. 1. A. Schematisierte Darstellung des Vorderendes des Zentralnervensystems von *Lumbricus terrestris*. Die dorsale, sekretorische Zone des Cerebralganglions ist dichter punktiert. B. Zellbild aus der sekretorischen Zone (Färbung Eosin-Lichtgrün): a, Gewöhnliche, mit Lichtgrün gefarbte Ganglienzellen. b, Mit Eosin rot gefarbte Zellen. c, Grünlich-violette Übergangsformen. (Orig.)

Tropfen von kolloidartigem Charakter. Mit Säurefuchsin färben sich diese Gebilde ebenfalls rötlich (vgl. dazu die auf S. 198 beschriebenen Endstadien der Sekretion im Nucleus praeropticus von *Cristiceps argentatus*).

Die unter (2) und (3) beschriebenen Ganglienzelltypen stehen untereinander in engerer Lagebeziehung als zu Typus 1. Sie bilden einen Bestandteil der im Nereis-gehirn am meisten kaudal liegenden Zellgruppen. Wahrscheinlich stellt das 2.

Stadium die Vorstufe für Stadium 3 dar. Dagegen kann noch nicht angegeben werden, in welcher Beziehung die spindelförmigen Zellen (Typus 1) zu den beiden übrigen sekretorischen Elementen stehen.

Über die sekretorischen Vorgänge im Zentralnervensystem der anderen untersuchten Wurmer kann noch kein abschliessendes Urteil abgegeben werden. Das Cerebralganglion von *Lumbricus terrestris* enthält in dem auf Fig. 1 A durch dichte Punktierung hervorgehobenen, dorsalen Bezirk auffällige Zellbilder (Fig. 1 B). In Präparaten, die nach Collin-Florentin mit Eosin und Lichtgrün gefärbt wurden, findet man neben den gewöhnlichen, grün erscheinenden Ganglienzellen (Fig. 1 B, a) solche, die sich mit Eosin kräftig rot imprägnieren (Fig. 1 B, b). Dazwischen finden sich Übergänge in Gestalt von grünlich-violett gefärbten Zellen (Fig. 1 B, c). Die mit Eosin färbbaren Zellen nehmen auch im Van Gieson-Präparat das Säurefuchsin stark an und unterscheiden sich damit schon bei schwacher Vergrößerung von ihren Nachbarzellen. In stark differenzierten Eisenhämatoxylinpräparaten (nach Heidenhain) halten die gleichen Zellen den Farbstoff am ausgiebigsten zurück und heben sich so von der nahezu farblosen Umgebung durch ihre schwarze Farbe deutlich ab. Ähnliche, im Van Gieson-Präparat tief rot gefärbte Ganglienzellen finden sich auch beiderseits im Cerebralganglion von *Hirudo medicinalis*. Bei Beobachtung mit Immersion erscheint das ganze Plasma erfüllt von einer äusserst fein gekörnten, rot gefärbten Masse. Trotz der geschilderten Verschiedenheiten dürfte es sich bei den bisher untersuchten Würmern um grundsätzlich die gleichen Zellformen, aber in verschiedenen Zustandsbildern der sekretorischen Tätigkeit handeln.

(2) MOLLUSCA

Die schönsten im Zentralnervensystem der Wirbellosen überhaupt gefundenen Sekretionsbilder zeigen die Opisthobranchier *Aplysia* und *Pleurobranchaea*. Auf einem nahe der Oberfläche gelegenen Flachschnitt durch das Cerebralganglion von *Aplysia limacina* (vgl. Fig. 2 A) finden wir symmetrisch zur Mittellinie zwei wohl abgegrenzte Zellgruppen. Schon bei schwacher Vergrößerung fallen diese im Van Gieson-Präparat durch ihre intensive Rotfärbung auf. Bei starker Vergrößerung erscheinen die betreffenden Zellen mit zahlreichen kleinen, leuchtend roten Tropfen angefüllt. Mit Eisenhämatoxylin nach Heidenhain färben sich diese Tröpfchen tief schwarz. Fig. 2 B zeigt eine solche Zelle bei starker Vergrößerung. Häufig liegt, wie es hier dargestellt ist, die Hauptmasse der verschiedenen grossen Sekrettropfen in den Randpartien der Zelle, um den Zellkern einen schmalen Bereich freilassend. Einen Teil der Zelle nimmt nicht selten ein nahezu farbloses, vielfach Vakuolen enthaltendes Maschenwerk ein, in dem sich keine Sekrettropfen finden. Es ist wohl anzunehmen, dass diese zu irgend einem Zeitpunkt (vielleicht erst bei der Fixierung) aus dem maschenartigen Bereich herausbefördert wurden. Die geschilderten Zellelemente unterscheiden sich ausser durch die fuchsinophilen Einschlüsse in nichts von den gewöhnlichen Ganglienzellen ihrer Umgebung. Insbesondere ist hervorzuheben, dass der Kern in keinem der untersuchten Fälle degenerative Veränderungen zeigt. Charakteristisch für die Kerne der sezernieren-

den Zellen ist nur, dass ihre Membran gelegentlich an einer Seite aufgelöst erscheint. Dies könnte im Sinne einer Beteiligung des Kerns an der Sekretion gedeutet werden.

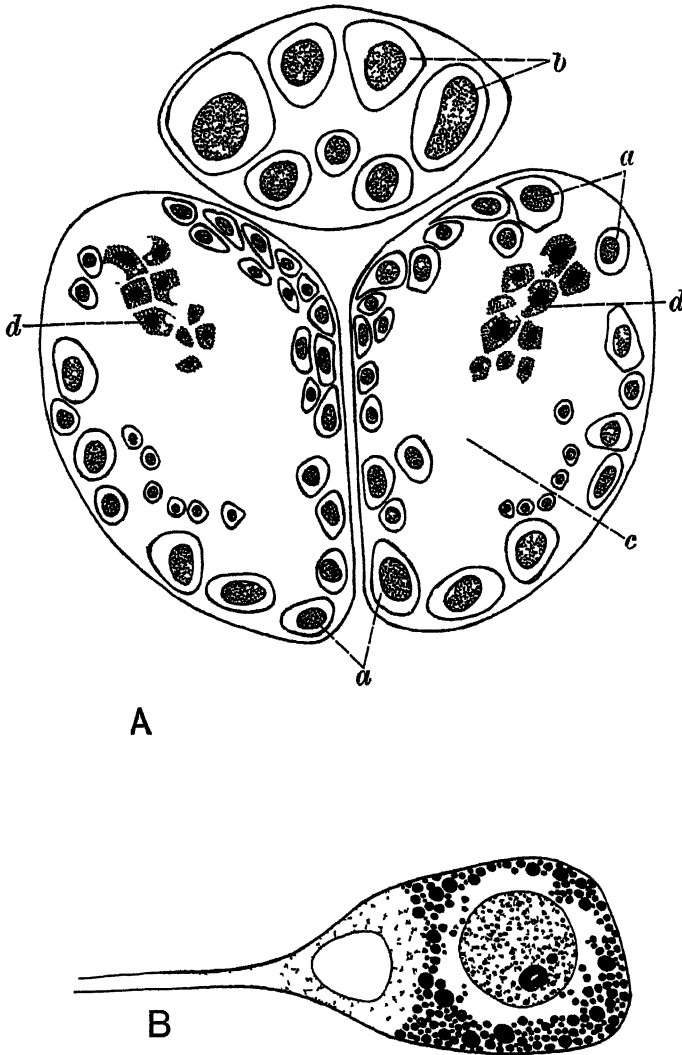


Fig. 2. A. Flachschnitt durch das Cerebralganglion von *Aplysia limacina* (schematisiert). *a*, Kleine und mittelgrosse Ganglienzellen. *b*, Grosse Ganglienzellen (Riesenzellen). *c*, Punktsubstanz (Neuropil). *d*, Gruppe sezernierender Zellen. (Die hier schwarz angegebenen Granula erscheinen im Van Gieson-Präparat leuchtend rot.) (Nach B. Scharrer, 1935.) B. Sezernierende Ganglienzelle bei stärkerer Vergrösserung. In den Randpartien des Plasmas zahlreiche, verschieden grosse Sekrettröpfchen. In der Nähe des Zellfortsatzes lockeres Maschenwerk mit Vakuole. Eisenhämatoxylin nach Heidenhain. (Orig.)

Ausser im Cerebralganglion finden sich ähnliche sekretorische Ganglienzellen sowie leere Vakuolen auch in den hinteren Visceralganglien von *Aplysia*. In den Pedalganglien und in den vorderen Visceralganglien werden nur vereinzelte, ausser-

halb von Zellen gelegene, mit Säurefuchsin ebenfalls rot färbbare Tröpfchen angetroffen; in den Buccalganglien fehlen auch diese.

Im wesentlichen die gleichen auffälligen Erscheinungen in dem entsprechenden scharf umschriebenen Bereich innerhalb des Cerebralganglions zeigen *Aplysia depilans* und eine noch nicht bestimmte Form (*Aplysia* sp.) aus der Bucht von Luanda (Angola, Westafrika). Keines der bisher untersuchten Exemplare lässt die sezernierende Zellgruppe vermissen; sie ist auch bei jüngeren Tieren vorhanden.

Fig. 3 A zeigt einen Flachschnitt durch das Cerebralganglion von *Pleurobranchaea meckeli*. Die drüsig funktionierende Zone liegt hier im kaudalen Bereich des Cerebrovisceralganglions (in der Fig. 3 A) in symmetrischer Anordnung zur Mittellinie. Die Sekretprodukte erscheinen bei dieser Schnecke in verschiedener Gestalt. In bestimmten Riesenzellen (Fig. 3 A, a) ist nahezu der ganze Plasmabereich mit feinsten fuchsinophilen Körnchen angefüllt. Ihre Grösse ist in der Regel bedeutend geringer als bei *Aplysia*. Doch gleichen im übrigen diese sezernierenden Zellen den bei *Aplysia* beschriebenen mehr als die im Folgenden zu schildernden Formen der Sekretbildung in Gestalt geschichteter Kolloidmassen. Weiterhin finden wir die Kernmembran in den sezernierenden Riesenzellen von *Pleurobranchaea* nicht selten an einer Stelle aufgelöst (Fig. 3 A, a). Die feinen Sekretkörnchen und -tropfen werden nicht nur im Innern, sondern auch ausserhalb der Zellen beobachtet. Mehrfach wurde das in Fig. 3 A mit b bezeichnete Bild beobachtet: in der unmittelbaren Umgebung einer in ihrer Form auffälligen Ganglienzelle liegen kleinere Anhäufungen von Sekrettröpfchen. Fig. 3 B demonstriert den mehrfach beobachteten Fall der Bildung von Sekret aus feinsten Körnchen in einer Riesenzelle und den Abtransport grösserer Sekretkörner auf dem Wege des Nervenfortsatzes (vgl. S. 208).

Weit auffälliger ist bei *Pleurobranchaea* eine andere Art von Sekret. Es handelt sich um grosse, rundlich-ovale oder unregelmässige Massen. Ihre Anordnung ist blumenstraussartig; sie zeigen eine deutliche Schichtung und finden sich entweder im Innern von Zellen oder häufiger ausserhalb von diesen gelegen (Fig. 3 A, c). Im letzteren Falle sind sie von zarten Hüllen umgeben, von denen aus feine Ausläufer ins Innere des Ganglions zu gehen scheinen. Vielleicht stellen diese Hüllen die letzten Reste ehemaliger Ganglienzellen dar, die ähnlich wie bei *Nereis* (vgl. S. 187) bei der Bildung des Sekretes zugrunde gegangen sind. Mit dem Van Giesonschen Säurefuchsin-Pikrinsäuregemisch färben sich diese Sekretmassen verschieden. Auf ein gelbes Zentrum folgt in der Regel eine rote Zwischenzone und schliesslich eine blassrote Aussenzone. Wahrscheinlich werden die Komponenten des angewandten Farbgemisches bei der Differenzierung von den einzelnen Schichten der Sekretkonkremente in verschieden starkem Masse zurückgehalten. Dies deutet auf eine verschiedene Konsistenz der einzelnen Schichten hin, muss aber nicht für ihre verschiedene stoffliche Zusammensetzung sprechen.

Bei den weiteren, bisher untersuchten Tectibranchiern *Tiedemannia*, *Cavolinia* und *Clione* konnten bisher keine sekretorischen Vorgänge im Zentralnervensystem beobachtet werden. Das Gleiche gilt für *Phyllirhoë bucephalum* unter den Nudibranchiern. Dagegen zeigen *Tethys*, *Doris* und *Aeolis* auffällige Bilder, die auf Grund der im Voranstehenden geschilderten Befunde als sekretorisch gedeutet

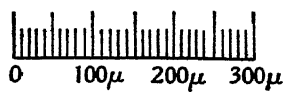
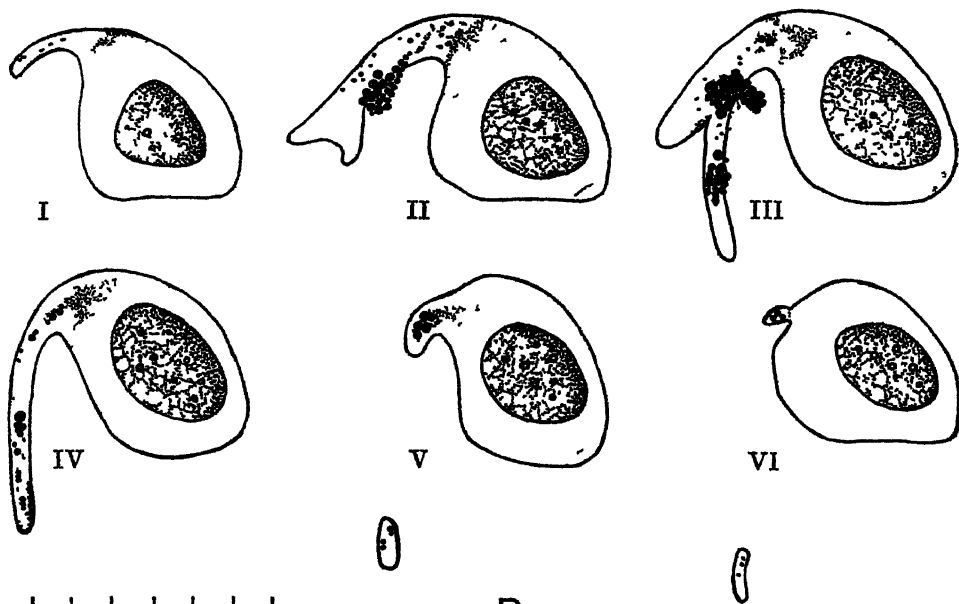
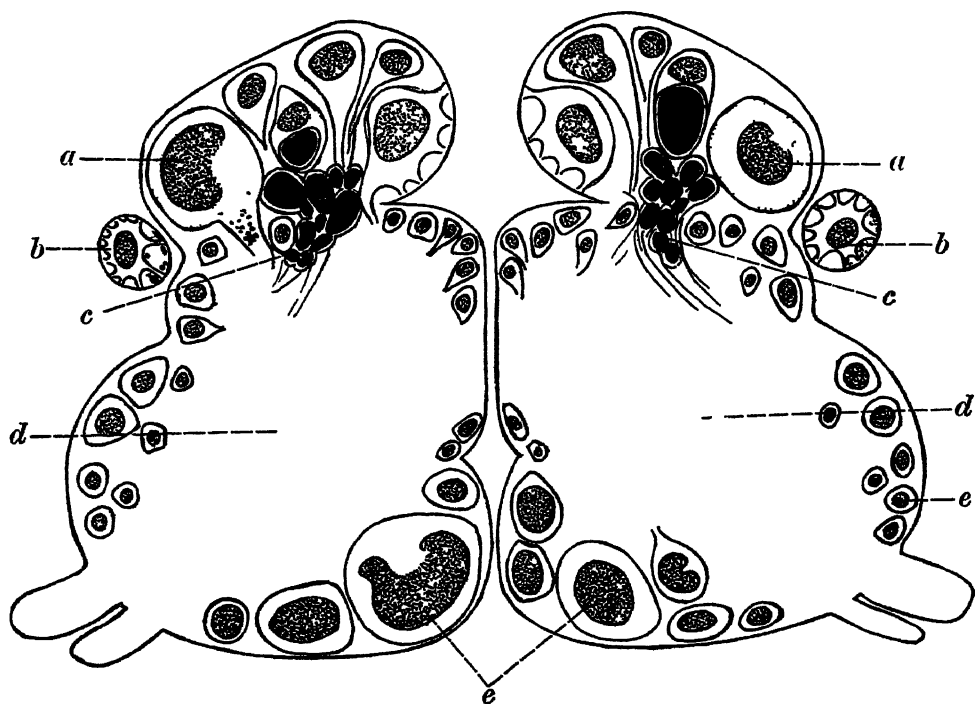


Fig. 3

werden dürfen, wenngleich sie an Deutlichkeit hinter *Aplysia* und *Pleurobranchaea* zurückstehen. Auf Einzelheiten soll hier verzichtet werden, da die Untersuchungen über diese Arten noch nicht abgeschlossen sind. Auch über die untersuchten Prosobranchier kann noch kein endgültiges Urteil abgegeben werden. Hier sprechen ebenfalls einige histologische Tatsachen für eine sekretorische Funktion einzelner Ganglienzellen.

Dagegen konnten sezernierende Ganglienzellen bei keiner der untersuchten vier Arten von Pulmonaten nachgewiesen werden. Ebenso negativ verlief auch die Untersuchung bei zwei Vertretern der Cephalopoden (*Sepia*, *Octopus*) und bei den Amphineuren *Chiton marginatus* und *C. discrepans*.

(3) ARTHROPODA

(a) Crustacea

Unter den Arthropoden liegen die ausgedehntesten Untersuchungen an den Crustaceen vor. Hanström (1931, 1933, 1934 a, b, c) untersuchte das Zentralnervensystem von Vertretern aller Hauptgruppen der Crustaceen. Sekretorisch tätige Nervenzellen konnten aber nur bei einer Reihe von Decapoden und Stomatopoden, sowie bei einem Vertreter der Anomotraca festgestellt werden. Bei den Crustaceen sind die sekretorischen Nervenzellen—wenn überhaupt—in einem von Hanström als Organ X bezeichneten Bereich anzutreffen. Diese "inkretorische Gehirndrüse" bildet einen Teil der Nervenmasse des Augenstiels und liegt zwischen der zu den primären Sehzentren gehörenden Medulla externa und den Corpora pedunculata, die bei den Crustaceen nach der Peripherie verlagert sind. Vorkommen und Ausbildung des Organs X sind bei den einzelnen Gruppen der Crustaceen verschieden. Während es bei allen untersuchten Stomatopoden nachgewiesen werden konnte, tritt es bei den Decapoden weniger regelmässig auf. Bei den *Macrura natantia* (Garneelen) fehlt das Organ unter der grossen Zahl der untersuchten Arten nur bei *Penaeus* sp. und bei *Benthescymus Bartletti*, während es bei der Gattung *Amphion* rudimentär gefunden wurde. Alle drei untersuchten Vertreter der *Macrura reptantia* lassen jedoch ein Organ X vermissen und bei den Anomura findet es sich nur bei *Eupagurus* und sehr schwach ausgebildet bei einer Larve von *Munida*. Ebenso ist das Organ bei der Mehrzahl der bisher untersuchten Brachyura schwach oder gar nicht ausgebildet. Bei *Pandalus borealis* und bei einer Reihe anderer Garneelen besteht das Organ X aus mehreren, traubenförmigen Lappen, die dem von der Medulla terminalis in der Nahe der Corpora pedunculata ausgehenden Nerven aufgelagert sind. Jeder Lappen ist wie das Zentralnervensystem selbst von einer dünnen Schicht bindegewebiger Fasern umgeben. In den

Fig. 3 A. Flachschnitt durch das Cerebrovisceralganglion von *Pleurobranchaea meckels* (schematisiert). a, Riesenzellen mit rotgefärbten (van Gieson) Granula; Kernmembran z. T. aufgelöst b, Ganglienzellen mit Sekrettröpfchen in ihrer Umgebung c, Gruppe geschichteter Konkreme in blumenstraussartiger Anordnung. d, Punktsubstanz (Neuropil). e, Ganglienzellen. B Riesen-Zelle aus dem Cerebralganglion von *Pleurobranchaea meckels* im Zustand der Sekretproduktion. Die Figuren I–VI stellen 6 aufeinanderfolgende, 20 μ dicke Schnitte der gleichen Zelle dar. Im Zellplasma liegen feinste, fuchsinophile Körnchen. Im Nervenfortsatz wandert das Sekret in Form grosserer Tröpfchen ab. (Nach B. Scharier, 1935)

nach Bouin fixierten und mit Hämatoxylin und Eosin gefärbten Schnittpräparaten gleichen die völlig runden Kerne der X-Zellen mit ihren grossen Nucleolen und reichlichem Chromatin denen der mittelgrossen Ganglienzellen. Die Sekretionsprodukte der X-Zellen treten als kleine, eosinophile, runde Tropfen oder als konzentrisch geschichtete, unregelmässige, in grossen Vakuolen gespeicherte Ballen in Erscheinung. Dazwischen finden sich grosse, leere Vakuolen, die offenbar früher von einer Substanz erfüllt waren, die bei irgend einer Gelegenheit herausbefördert wurde. Im einzelnen lassen sich unter den Garneelen manche Unterschiede feststellen. Bei *Acanthecephyra* überwiegen in den von Hanström untersuchten Exemplaren die grossen leeren Vakuolen, bei anderen Arten, z. B. bei *Virbius* finden sich schlechter ausgebildete Vakuolen, dafür aber häufiger geschichtete Ballen in den Zellen. Hanström vermutet, dass die eosinophilen Sekrettröpfchen eine Vorstufe zu der in den Vakuolen befindlichen Substanz darstellen.

Bei den Stomatopoden hat das Organ X die gleiche Lage im Augensiel wie bei den Decapoden. Sein Bau ist bei den vier von Hanström untersuchten *Squilla*-Arten im grossen und ganzen derselbe. Bei *Squilla mantis*, wo das Organ erstmals (1931) festgestellt wurde, und bei *S. oratoria* ist es etwas höher differenziert als bei den beiden anderen Arten. Hier ist es—ähnlich wie bei *Pandalus*—traubenförmig, während es bei *Squilla eusebia* und *S. desmaresti* nur einfach strangförmig gebaut ist. Bei *Squilla eusebia* sind die X-Zellen in einer einzigen, einzelligen Schicht angeordnet; sie grenzen mit ihrer Innenseite an einen feinen Nerven und an ein Blutgefäss. Die dieser Seite zugewandten Zellpartien sind mit Sekret erfüllt, während die von blassgefärbtem Plasma umgebenen, ganz denen der angrenzenden Ganglienzellen gleichenden Kerne an der Aussenseite der Zellen liegen. Das in grosser Menge vorhandene Sekret erscheint bei den Stomatopoden in der Regel in Form von runden oder rundlichen, verschieden grossen, tropfenartigen Gebilden. Die kleinsten Tröpfchen liegen in der Umgebung der Zellkerne, wo sie nach Hanström offenbar gebildet werden um dann später zu grösseren Tropfen zusammenzufließen. Diese trifft man entweder in Vakuolen an oder in einem blassen Plasmabereich. Bei *Squilla eusebia* fand sich in einzelnen Fällen (wie bei manchen Garneelen) eine Andeutung von konzentrischer Schichtung der Sekrettropfen. Nach ihrem färberischen Verhalten handelt es sich bei den geschilderten Sekretsubstanzen nicht um Lipide. Sonst kann über ihre chemische Zusammensetzung noch nichts ausgesagt werden.

Bei einem Vertreter der Anomotraca, *Anaspides tasmaniae*, konnte Hanström ebenfalls ein wohl ausgebildetes Organ X feststellen. Es liegt hier am vorderen ventralen Rand des Augensiels, dicht unter der Hypodermis und grösstenteils ausserhalb der Ganglienzellenschicht der Medulla terminalis. Ebenso wie bei den Decapoden und Stomatopoden wird es vom Nervus innominatus (Hanström) versorgt. Wie bei diesen besteht das Organ X von *Anaspides* aus mehreren runden, völlig an Ganglienzellen erinnernden Zellelementen. Das Sekret findet sich in Form feiner, in Vakuolen gespeicherter eosinophiler Tröpfchen oder deutlich geschichteter Schollen.

(b) Myriapoda und Insecta

Bei zwei sehr grossen, im äquatorialen Westafrika gesammelten *Juliden*-Arten liessen sich im Zentralnervensystem keine sezernierenden Ganglienzellen feststellen. Was die Gruppe der Insekten anbelangt, so kennen wir bis jetzt nur eine Beobachtung, die mit den bei den übrigen Wirbellosen geschilderten Befunden weitgehende Übereinstimmung zeigt. Weyer (1935) fand im Bienenhirn regelmässig ein unpaares bzw. aus einer paarigen Anlage verschmolzenes, traubiges "Organ", dessen Zellen sich von den umgebenden, gewöhnlichen Ganglienzellen deutlich unterscheiden. Die Lage dieser Zellgruppe ist stets median im Proto-

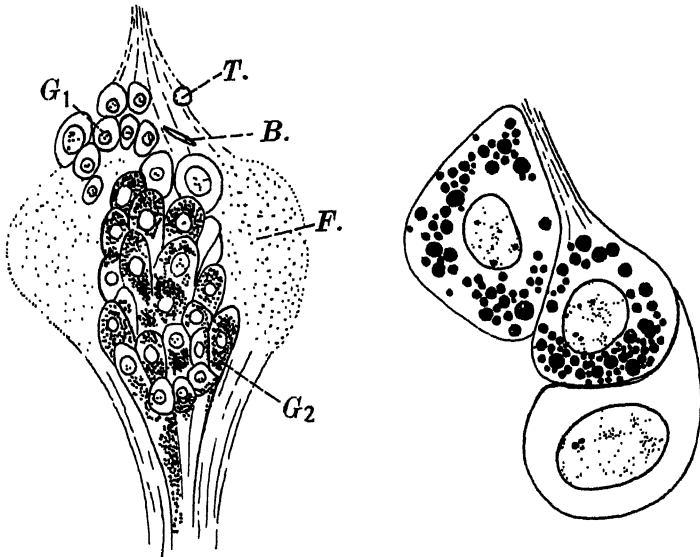


Fig. 4. Drüsen-Nervenzellen im Gehirn von *Apis mellifica* (Arbeiterin). Links bei schwächerer, rechts bei stärkerer Vergrösserung. B.=Bindegewebszelle. F.=Fasermasse im Querschnitt. G_1 =Gewöhnliche Ganglienzellen. G_2 =Ganglienzellen mit Sekrettröpfchen. T.=Trachee. (Nach Weyer, 1935; für die freundliche Überlassung der Figuren sind wir Herrn Dr. Weyer in Hamburg sehr zu Dank verpflichtet.)

cerebrum, vor dem Zentralkörper und zwar in gleicher Höhe mit demselben zwischen den inneren Wurzeln der Corpora pedunculata. Dieses Organ gehört anatomisch wohl zur Pars intercerebralis und besteht wie diese aus sehr grossen, nervösen Elementen (Fig. 4). Diese enthalten in ihrem Cytoplasma reichlich sekretartige Tropfen von verschiedener Grösse, die sich sehr kräftig mit Hämatoxylin (nach Delafield und Heidenhain), weniger deutlich mit Plasmafarbstoffen imprägnieren. Ähnlich wie bei den Opisthobranchiern (*Aplysia*, *Pleurobranchaea*) liegen die Sekrettröpfchen vielfach an der Austrittsstelle des Nervenfortsatzes gehäuft; in der Regel befinden sich in der direkten Umgebung des Kerns und in einer schmalen Randzone der Zelle keine Tropfen. Die Sekretgranula von *Apis mellifica* geben keine Fettreaktion und lassen sich nicht wie Glycogen färben (Jodreaktion nach Langhans). Gegen die Deutung als pathologische Zellbilder

spricht hier ebenso wie bei den Schnecken und Würmern das regelmässige Vorkommen der Drüsenzellengruppe—Weyer untersuchte einige hundert Schnittserien—und ihre deutlich abgegrenzte Lage im Bienengehirn. Bei frisch geschlüpften und sehr alten Arbeitsbienen liessen sich die tropfenförmigen Einschlüsse in den Nervenzellen nicht mit Sicherheit nachweisen. Am besten entwickelt ist das Organ bei jüngeren Trachtbienen, weniger deutlich bei der Bienenkönigin, am wenigsten bei der Drohne. In einigen Zellen des Organs fanden sich manchmal nur wenige Sekrettropfen, in anderen sehr viele. Ausserhalb der Nervenzellen wurden sie bei *Apis mellifica* niemals angetroffen. Über die Entstehungsweise der Tropfen und die Cytologie der Drüsen-Nervenzellen bei der Biene liegen noch keine Untersuchungen vor.

III. WIRBELTIERE

(1) CYCLOSTOMATA

Über Drüsen-Nervenzellen im Gehirn der Cyclostomen liegen keine Untersuchungen vor. Speidel (1922) fand die von ihm im Rückenmark von Selachiern, Ganoiden und Teleostiern beschriebenen grossen Drüsen-Nervenzellen beim Neunauge (*Petromyzon*) nicht.

(2) PISCES

(a) *Elasmobranchii*

Im Gehirn der Selachier und zwar im Zwischenhirn finden sich sezernierende Ganglienzellen. Bei Rochen liegt über dem Chiasma opticum ebenso wie bei den Knochenfischen der Nucleus praeopticus als eine Gruppe grosser Nervenzellen, die durch die periphere Lage grober Nisslschollen ausgezeichnet sind. Bei *Raja asterias* (Neapel, 1934; Bouin, Celloidin, Weigerts Hämatoxylin-Van Gieson) weisen die Zellen des Nucleus praeopticus teils kleine, stark mit Säurefuchsin rot gefärbte Granula im Bereich der Nisslschollen auf, teils treten einzelne grosse Tropfen, wie man sie auf den gleichen Schnitten im Zwischenlappen der Hypophyse findet, in Erscheinung und schliesslich kann das Plasma mancher Zellen im ganzen von einer feinkörnigen, rot gefärbten Substanz erfüllt sein. Die Verhältnisse stimmen völlig mit den noch zu schildernden Bildern bei den Knochenfischen überein. Bei Scyllium konnte keine so deutliche Differenzierung des Nucleus praeopticus festgestellt werden und es wurden auch keine sekretorisch tätigen Zellen beobachtet.

Im Rückenmark von Rochen hat Dahlgren (1914) motorische Nervenzellen als sekretorisch tätig beschrieben. Er fand in verschiedenen Abschnitten des Rückenmarks von 11 Arten ungewöhnlich grosse, unregelmässig gestaltete Vorderhornzellen mit gelappten und verzweigten, chromatinreichen Kernen. Diese Zellen enthalten Vakuolen, deren Inhalt in Form homogener Granula aus dem Zelleib ausgestossen und vermutlich auf dem Blutweg abtransportiert wird. Ein derart ungewöhnliches Verhalten veranlasste Dahlgren die nervöse Herkunft dieser Zellen in Zweifel zu ziehen. Beim Studium der Entwicklung der Zellen stellte sich aber heraus, dass sich diese grossen sekretorischen Nervenzellen schon auf frühem

Embryonalstadium aus motorischen Vorderhornzellen entwickeln, also nervöser Herkunft sind. Die Untersuchungen wurden von Speidel (1919, 1922) an *Raja ocellata*, *R. laevis*, *R. erinacea* und *R. radiata* fortgeführt. So wurden bei *Raja ocellata* 120 Wirbel gezählt; von der Höhe des 74. Wirbels bis zur Schwanzspitze wurden die in Frage stehenden Zellen in einer Gesamtzahl von etwa 600 gefunden. Sie treten hier in der hinteren Hälfte des Rückenmarks beiderseits vom Zentralkanal durch ihre unregelmässige Gestalt und ihre ausserordentliche Grösse (z. B. $300\mu : 200\mu : 176\mu$), die das zofache einer motorischen Vorderhornzelle beträgt, eindrucksvoll in Erscheinung. Der Zellkern ist vielfältig verzweigt und gelappt (Fig. 5). Nisslschollen und Neurofibrillen konnten in diesen Zellen nicht fest-

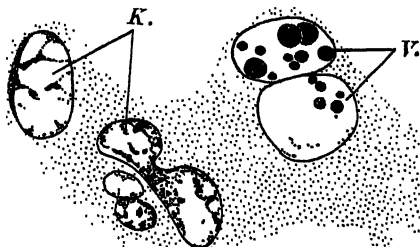


Fig. 5. Zelle aus einem Längsschnitt durch das Rückenmark von *Raja laevis*. Mehrere Anschnitte des gelappten Kerns (K). Zwei Vakuolen mit Sekretgranula (V). (Nach einer Mikrophotographie von Speidel (1919).)

gestellt werden. Auffällig ist auch die ausserordentlich reiche Gefässversorgung der Zellen, die jeweils mit 4–5 Kapillaren in Kontakt stehen. Bisweilen ist auch ein Gefäss ganz vom Zellplasma umschlossen.

Das Cytoplasma dieser Zellen enthält nun weiterhin Einschlüsse in Form von Granula und Tropfen sowie Vakuolen verschiedener Grösse (Fig. 5). Diese entwickeln sich so, dass erst zahlreiche kleine Vakuolen im Zellplasma auftreten, die nach und nach zu einigen wenigen grösseren Hohlräumen zusammenfliessen. In ihrem Inneren, das zunächst keinen färbbaren Inhalt oder nur netzförmige, mit sauren Farbstoffen färbbare Niederschläge erkennen lässt, erscheinen später Granula, die an Zahl und Grösse zunehmen. In manchen Vakuolen findet man auch nur einige wenige oder vereinzelte sehr grosse Granula. Über das histochemische Verhalten dieser Granula vgl. S. 209.

(b) *Teleostomi*

(1) *Dipnoi*.

Im Zwischenhirn von *Lepidosiren paradoxa* (ein lebendfrisch fixiertes Exemplar) konnten keine Anzeichen einer sekretorischen Tätigkeit in der Regio praeoptica festgestellt werden.

(2) *Rhomboganoidei*.

Beim nordamerikanischen Kaimanfisch (*Lepidosteus osseus*) fand Speidel (1922) die gleichen grossen Drüsenzellen im Rückenmark wie bei den Rochen. Sonst liegen keine Untersuchungen an Ganoiden, im besonderen über das Vorkommen von Drüsen-Nervenzellen im Gehirn vor.

(3) *Teleostei*.

Bei den Teleostiern gibt es fünf sekretorisch tätige Zellgruppen. In der Richtung von oral nach kaudal sind dies:

Nucleus nervi terminalis,
Nucleus praepopticus, pars magnocellularis,
Nucleus lateralis tuberis,
Die Mittelhirngruppe (Scharrer, 1932 b),
Die Speidelsche Rückenmarksdrüse.

Nucleus nervi terminalis. Einige grosse Zellen des Kerns des Nervus terminalis (Brookover, 1910) weisen bei *Xiphias gladius* (Scombridae) in ihrem Inneren Kolloidtropfen auf. Bei *Tetrodon lagocephalus* (Plectognathi) sind die Zellen durch ausserordentlich vielgestaltige Kerne, die vielfach den im Rückenmark der Rochen beschriebenen entsprechen (Fig. 5), ausgezeichnet. Da die Zellen auch intrazelluläre Kapillaren besitzen, ist es wahrscheinlich, dass sie bei *Tetrodon* ebenfalls sekretorisch tätig sind, obwohl hier keine Kolloidtropfen gefunden wurden. Freilich stand nur wenig Material für die Untersuchung zur Verfügung und in diesem Falle besagt eine negative Feststellung erfahrungsgemäss wenig.

Nucleus praepopticus. Was den grosszelligen Anteil des Nucleus praepopticus anbelangt (Fig. 6 A), so kommt es hier bei vielen Fischen zur Ausbildung einer richtigen Zwischenhirn-drüse. Dies gilt z. B. in besonders ausgesprochenem Masse für den Blenniiden *Cristiceps argentatus* (Scharrer, 1935). Hier trifft man nebeneinander Zellen, deren Plasma in gut differenzierten Eisenhämatoxylin-Präparaten feinste, bei stärkster Vergrösserung eben sichtbare, blauschwarz gefärbte Körnchen enthält. Von solchen kleinsten Körnchen führen alle Zwischengrössen bis zu auffälligen, mit Eisenhämatoxylin schwarz oder mit Säurefuchsin rot gefärbten Tropfen wie man sie in ähnlicher Form in der Hypophyse der Fische und anderer Wirbeltiere als "Kolloid" findet (Fig. 6 B). Während die kleinen Körnchen oder einzelne mittelgrosse Tropfen noch frei im Plasma der Zelle zu liegen pflegen, bildet sich bei gesteigerter Kolloidproduktion eine Vakuole, in der eine Anzahl untereinander oft gleich grosser Tropfen liegt. Derartige Vakuolen können eine beträchtliche Grösse erlangen und die so entstehenden Gebilde erinnern in nichts mehr an Ganglienzellen, wohl aber bieten sie das Bild von Drüsenzellen. Sie verwandeln sich schliesslich in grosse Blasen, die in ihrem Inneren einige Sekrettropfen enthalten und an deren Wand der meist pyknotische Kern zusammen mit einem Plasmarest liegt (vgl. *Nereis*, S. 187). Daneben finden sich Zellen mit kleinen geschrumpften Vakuolen am Rande des Plasmaleibes. Offenbar können die Zellen mehrmals Sekret produzieren und abgeben ohne zugrunde zu gehen. Die

färbbaren Körnchen und Tropfen, die den Inhalt der grossen und kleinen Vakuolen bilden, verschwinden wahrscheinlich aus diesen, indem sie aufgelöst werden. Man

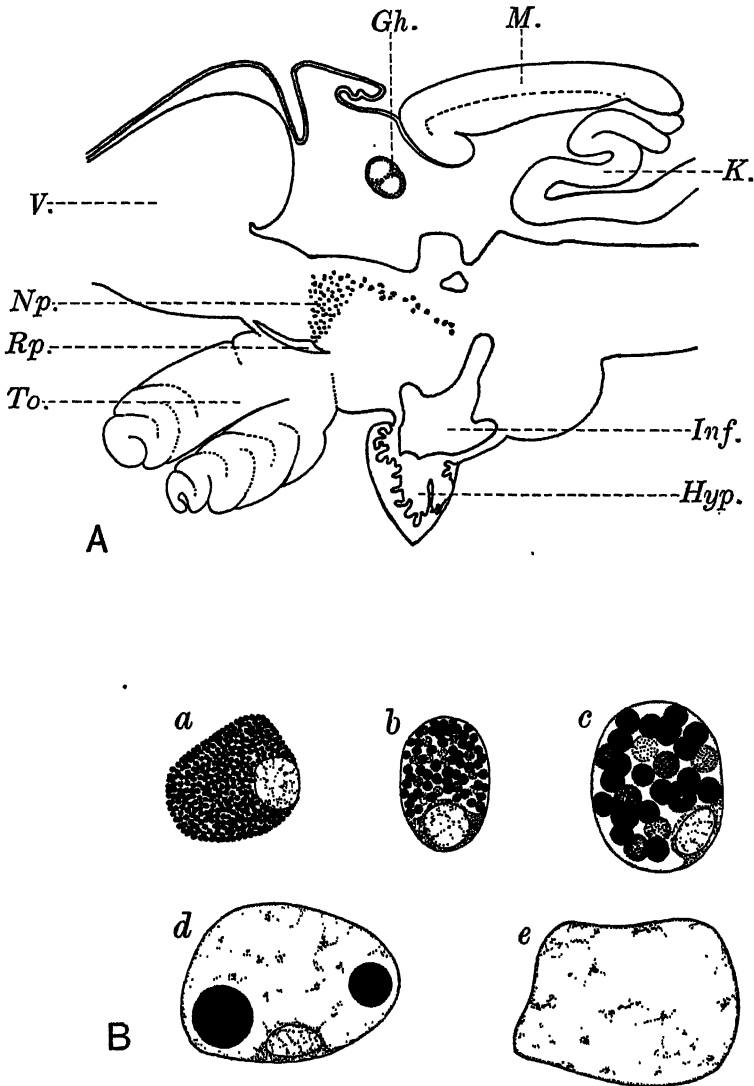


Fig. 6. A. Sagittalschnitt durch das Gehirn eines Knochenfisches. Gh.=Ganglion habenulae. Hyp.=Hypophyse. Inf.=Infundibulum. K.=Kleinhirn. M.=Mittelhirn. Np.=Nucleus praeopticus. Rp.=Recessus praeopticus. To.=Tractus opticus. V.=Vorderhirn. (Nach E. Scharrer, 1932 a.) B. a-e, Verschiedene Stadien der Sekretbildung im Nucleus praeopticus von *Cristiceps argentatus*. (Nach E. Scharrer, 1934 b.)

sieht immer nur intakte Vakuolenwände, das Sekret scheint also nicht durch Einreissen der aufgeblähten Zellwände entleert zu werden.

Cristiceps stellt einen extremen Fall von Umwandlung der Nervenzellen in Drüsenzellen im Bereich des Zwischenhirns dar. Bei den meisten übrigen Knochen-

fischen kommt es nicht zu einem derart ausgeprägten Verlust des nervösen Charakters der in Frage stehenden Zellen. Diese weisen zwar Nisslschollen und Zellfortsätze auf, weichen aber durch die Einlagerung von Sekretgranula und durch die ungewöhnliche Polymorphie der Kerne von der Norm der Ganglienzelle doch weitgehend ab. Beispiele für dieses Verhalten stellen *Phoxinus laevis*, *Fundulus heteroclitus*, *Tinca vulgaris* und andere dar. Der Charakter der Zellen wird dabei durch die verschiedene Art der Sekretbildung in sehr verschiedenem Ausmass verändert. Bei *Phoxinus* trifft man nur selten intrazellulär liegendes Kolloid an und dann meist nur in Form kleiner Granula. Offenbar werden grössere Tropfen rasch ausgestossen und man findet deshalb in der Regel massenhaft Kolloid zwischen den Zellen. Bei *Fundulus* und *Tinca* ist dagegen intrazellulär liegendes Kolloid häufiger anzutreffen. Aber auch diese beiden Vertreter unterscheiden sich im Gesamtbild der Zwischenhirndrüse wieder deutlich voneinander: bei *Tinca* liegt das Kolloid in Form von Haufen kleiner Tropfen vor allem in den lateralen Partien des Nucleus praepopticus, während es bei *Fundulus* zu grossen runden Tropfen zusammenfliesst. Aus diesen kann das Sekret offenbar herausgelöst werden, sodass schliesslich nur noch Blasen mit körnigem Inhalt übrig bleiben. Wieder anders ist das Bild bei *Tetrodon lagocephalus*. Hier zeigt fast jede Zelle einen marginalen Kolloidstreifen. Bei einer südafrikanischen Welsart dagegen, *Galeichthys feliceps*, tragen die Zellen grosse Kolloidsäcke, die etwas an die Lipoidsäcke der Ganglienzellen bei der amaurotischen Idiotie der menschlichen Pathologie erinnern. Bei *Galeichthys* ist im übrigen die gleiche Beteiligung des Zellkerns an der Sekretion im Nucleus praepopticus zu beobachten wie in dem im Folgenden geschilderten Nucleus lateralis tuberis von *Tinca* (vgl. Fig. 7). So laufen zwar alle diese Bilder auf das Gleiche hinaus, nämlich die Bildung eines kolloidartigen Sekretes durch die Nervenzellen, aber die Art und Weise der Sekretion zeigt nicht unwesentliche Unterschiede.

Schliesslich wurden bei einer Anzahl von Knochenfischen im Bereich des Nucleus praepopticus nur typische grosse Nervenzellen angetroffen, die zunächst keine Kolloidbildung erkennen liessen. Dies gilt z. B. für *Salmo fario*, *Ameiurus nebulosus* und andere. Es ist aber anzunehmen, dass der Nucleus praepopticus bei allen Fischen sekretorisch tätig ist. Nicht immer freilich muss der Nucleus praepopticus in der Phase der Kolloidbildung angetroffen werden. Bei Untersuchung weniger Exemplare einer Art kann dann das Fehlen sekretorischer Vorgänge vorgetäuscht werden. Die Verhältnisse im Zwischenhirn der Knochenfische wurden in mehreren Untersuchungen (E. Scharrer, 1928, 1930, 1932 a, 1934 b, c, 1935) geschildert und es mag hier der Hinweis auf diese ja bekannten Tatsachen genügen.

Nucleus lateralis tuberis. Besondere Verhältnisse liegen im Nucleus lateralis tuberis vor. Bei den meisten der Fische, bei denen im Nucleus praepopticus eine deutliche Sekretion festgestellt werden konnte, zeigt auch der Nucleus lateralis tuberis auffällige Zellformen mit polymorphen Kernen, Kerneinschlüssen und Plasmavakuolen. Dies gilt z. B. für *Esox lucius*, *Tinca vulgaris*, *Perca fluviatilis*, *Crenilabrus pavo*, *Tetrodon lagocephalus* u. a. Dagegen lässt sich der Nucleus lateralis tuberis bei *Phoxinus laevis* und *Fundulus heteroclitus* überhaupt nicht

feststellen. Bei anderen Arten zeigt er keine histologischen Besonderheiten. Um so merkwürdiger ist aber sein Verhalten bei den zuerst genannten Formen, z. B. bei *Esox lucius*. Die Kerne weisen hier oft lange filamentartige Ausläufer auf, die sich bisweilen ein Stück weit in den Zellfortsatz erstrecken. Besonders bemerkenswert erscheint auch die Tatsache, dass diese Kernaussläufer gleichsam vom Ventrikel weggehen und alle nach lateral gerichtet sind (Fig. 7). Stets lassen sich verschiedene Stadien der Abschnürung von Kernsegmenten beobachten und nicht selten enthalten die Kerne eine Anzahl von Nucleolen verschiedener Gestalt. Die Verhältnisse bei der Schleie (*Tinca vulgaris*) fallen ganz und gar aus dem Rahmen dessen heraus, was man bisher von Nervenzellgruppen zu sehen gewohnt war. Auf's Deutlichste lässt sich bei der Schleie auch die Bildung des Sekretes innerhalb des Zellkerns in allen Phasen beobachten (Fig. 7). Ferner ist bei der Schleie der Nucleus lateralis tuberis in funktionelle Beziehung zur Hypophyse getreten und macht mit dieser jahreszeitliche Funktionsschwankungen mit. Während im Winter die Zellen des Nucleus lateralis tuberis ruhen, zeigen sie bei Sommertieren eine lebhaftere Sekretionstätigkeit. Weiterhin nehmen die Zellen des Nucleus lateralis tuberis auch Hypophysenkolloid auf und das Zwischenhirnkolloid wandert entlang den Neuriten der Zellen des Nucleus praeopticus von diesem zum Nucleus lateralis tuberis herab. Der Nucleus lateralis tuberis stellt bei der Schleie und wohl auch bei anderen Fischen die zentrale Stelle für die Verarbeitung des Hypophysen- und des Zwischenhirnkolloids dar und seine Beziehungen zum Nucleus praeopticus bilden gleichsam ein Modell eines Hypophysen-Zwischenhirnsystems.

Die Mittelhirngruppe. Eine weitere Zellgruppe, die wegen der auffälligen Gestalt ihrer Kerne schon Holmgren (1920) auffiel, findet sich im Mittelhirn. Ihre Lage wurde bereits früher beschrieben (E. Scharrer, 1932 b), wobei auch gezeigt wurde, dass bei der Elritze (*Phoxinus laevis*) in seltenen Fällen Sekretion in diesen Zellen beobachtet werden kann. Bei anderen Fischen konnten in dieser Zellgruppe niemals sekretorische Vorgänge beobachtet werden. Ihre Faserverbindungen und ihre Homologie mit anderen Kernen im Mittelhirn höherer Wirbeltiere sind noch unbekannt.

Die Speidelsche Rückenmarksdrüse. Speidel (1922) hat bei einer Anzahl von Knochenfischen die gleichen sekretorischen Zellen im Rückenmark gefunden wie bei den Rochen. Besonders schön ausgebildet und in grosser Anzahl finden sie sich in der terminalen Rückenmarksanschwellung bei der sogen. Sommerflunder (*Paralichthys dentatus*). Die Zellen gleichen durch die periphere Lagerung des vielfach verzweigten Kerns auf dem Schnitt in gewisser Weise den Riesenzellen in miliaren Tuberkeln. Es wurden hier Zellen gemessen von 240μ : 160μ : 110μ . Diese Zellen enthalten nur wenige Granula und weisen selten Vakuolen auf.

(3) AMPHIBIA

(a) Urodela

Bei den Urodelen ist die Zwischenhirnsekretion ausserordentlich unbedeutend. Nur selten findet man beim Salamander und bei den verschiedenen untersuchten

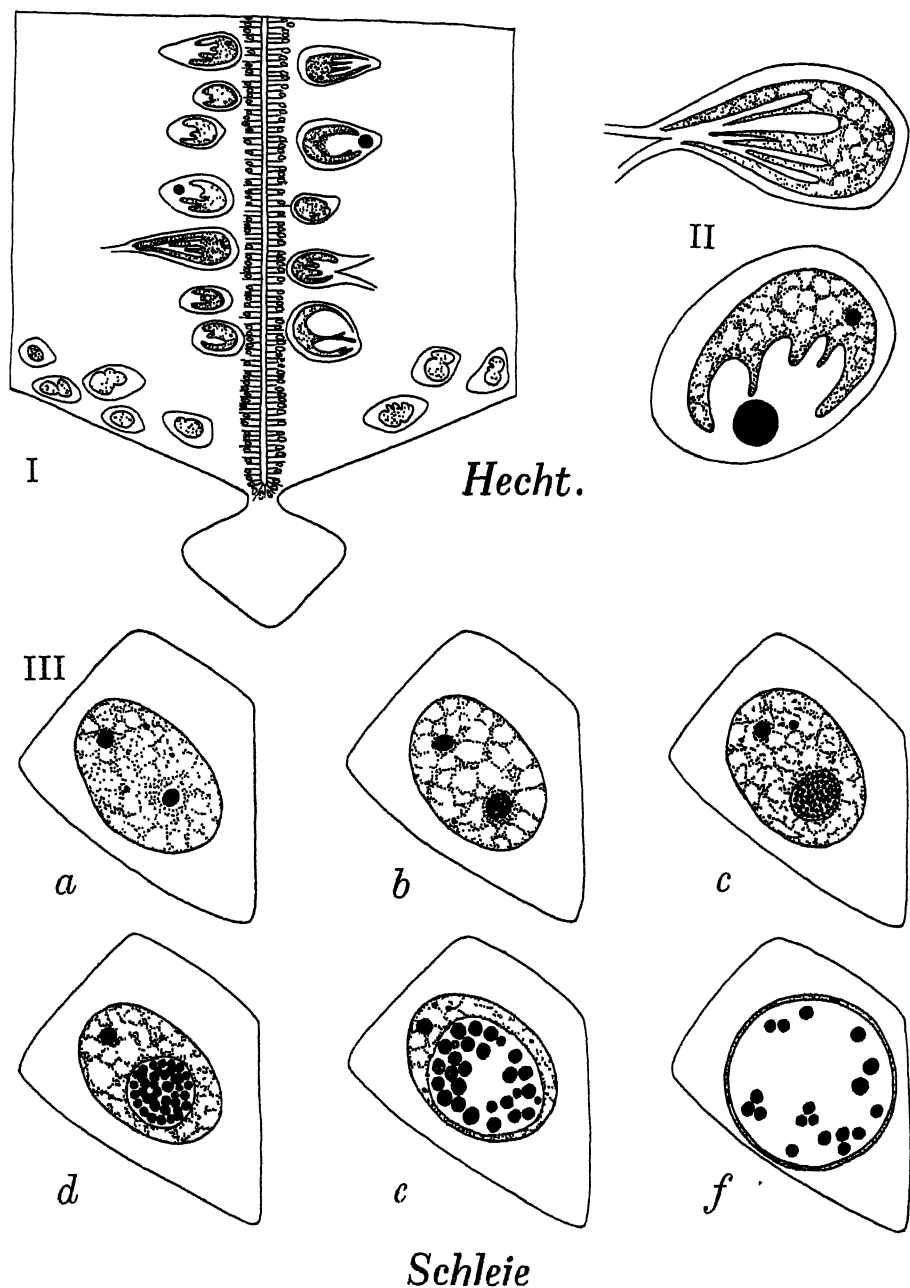


Fig. 7. I. Querschnitt durch den Nucleus lateralis tuberculi des Hechtes (*Esox lucius*). Die Kerne der Zellen sehen mit ihren Ausläufern vom Ventrikel weg. II. Zwei Zellen aus dem Nucleus lateralis tuberculi des Hechtes bei stärkerer Vergrößerung. Eine Zelle mit einem grossen Kolloidtropfen. III, a-f. Verschiedene Phasen der Kolloidbildung im Zellkern im Nucleus lateralis tuberculi der Schleie (*Tinca vulgaris*). (Nach E. Scharrer, 1934 c, verändert.)

Molcharten ganz feine, schwach färbbare Körnchen im Bereich des Nucleus praеоpticus, der im übrigen bei den Urodelen wohl ausgebildet ist.

(b) *Anura*

Während die Urodelen in der Geringfügigkeit der Sekretion übereinstimmen, sind die Verhältnisse bei den Anuren verschieden. Bei *Rana* und *Hyla* ist die Zwischenhirnsekretion ebenso schwach wie bei den Urodelen. Dagegen haben wir bei *Alytes*, *Bombinator* und *Pelobates* stets eine deutliche sekretorische Tätigkeit beobachten können. Die eindrucksvollsten Bilder bietet aber ohne Zweifel die Gattung *Bufo* (Fig. 8). Vor allem die südliche, etwas grössere Rasse von *Bufo*

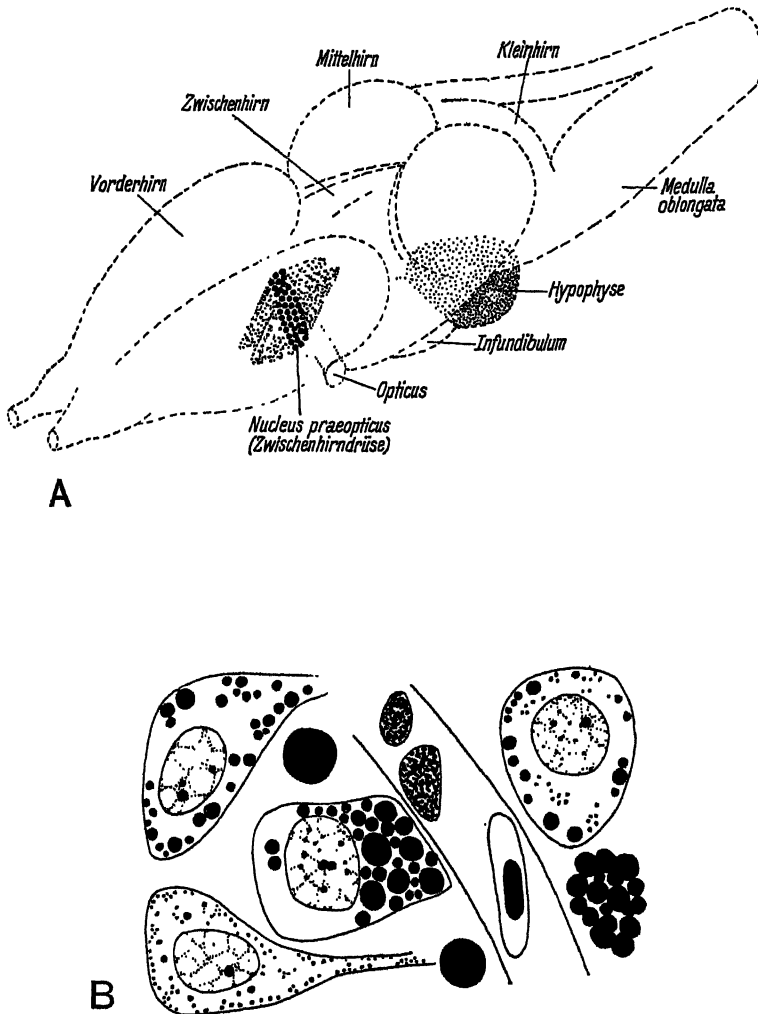


Fig. 8. A. Topographie der Zwischenhirnendrüse (Nucleus praеоpticus) im Gehirn der Erdkröte (*Bufo vulgaris*). (Nach E. Scharrer, 1934 a.) B. Drüsen-Nervenzellen und freies Sekret aus dem Nucleus praеоpticus der Kröte (*Bufo*). (Nach E. Scharrer, 1933 d.)

vulgaris zeigt im Zwischenhirn stets gewaltige Massen von Kolloid (Fig. 8 B) und der Nucleus praeropticus bietet bei der Kröte mehr das Bild einer Drüse als einer Nervenzellgruppe. Die Zellen enthalten oft so zahlreiche Kolloidtropfen, dass ausser dem Kern von der Zelle selbst nichts mehr zu sehen ist, diese vielmehr nach Art der Russelschen Maulbeerzellen gänzlich in der Kolloidbildung aufgegangen sind. Auch zwischen den Zellen finden wir grosse Massen von Kolloid in Gestalt traubenförmiger Klumpen und grosser Tropfen. Bemerkenswert ist des weiteren die scharfe Beschränkung der kolloidföhrnden Zellen auf den grosszelligen Bezirk des Nucleus praeropticus (Fig. 8 A). Wir werden auf die Verhältnisse bei der Kröte bei Gelegenheit der Erörterung der physiologischen Bedeutung der Zwischenhirndrüse sowie der Frage des Sekretionsvorganges zuruckzukommen haben.

(4) REPTILIA

(a) *Chelonia*

Im Hauptkern des Nucleus magnocellularis periventricularis fand Kurotsu (1935) bei *Dammonia* (*Geoclemys*) *subtrijuga*, einer in Java und Siam heimischen Schildkröte, viele mit Sekrettropfen verschiedener Grösse gefüllte Zellen. Die spindelförmigen oder ovalen Zellen liegen teils dicht an der Kapillarwand, teils sub- oder intraependymal. Kurotsu schliesst daraus, dass die Sekrete teils in den Liquor, teils in das Blut übertreten. In Weigert-Präparaten färben sich die Sekrettropfen mit Hamatoxylin schwarz, im Van Gieson-Präparat gelb. Darnach scheint es sich um durchaus mit den von uns an anderen Wirbeltieren erhobenen Befunden übereinstimmende Verhältnisse zu handeln, was auch Kurotsu betont.

(b) *Squamata*

Bei den Eidechsen und Geckonen findet sich im Bereich des sehr unansehnlichen, aus wenigen dürrtigen Zellreihen bestehenden Nucleus supraopticus in der Regel Kolloid in Gestalt von kleinen, mit Säurefuchsin stark farbbaren Tropfen. Bei *Tarentola mauretanica* wurden ebenso wie bei manchen Fischen im Zentrum der Kolloidtropfen stark lichtbrechende Kristalloide beobachtet. In den Zellen selbst, die mit ihren peripher liegenden Nisslschollen dem Typus der sekretorischen Zwischenhirnzellen entsprechen, wurden bei den untersuchten Tieren keinerlei Granula oder Tropfen gefunden.

(5) AVES

Von den Vögeln wurde eine Anzahl von Arten untersucht. Da aber jeweils nur ein Exemplar zur Verfügung stand, ist dem bisher stets gleichbleibend negativen Befund zunächst wenig Bedeutung zuzumessen. Immerhin hat auch Kurotsu (1935), der den Nucleus paraventricularis der Reptilien und Vögel untersuchte und auf die Zwischenhirnsekretion besonders achtete, nichts darüber mitgeteilt. Eine spezielle Untersuchung der Vögel wäre in dieser Hinsicht erwünscht.

(6) MAMMALIA

Bei den Säugern ebenso wie bei den niederen Wirbeltieren und den Wirbellosen bestehen bezüglich der Neurosekretion bei den einzelnen Arten und Gattungen unerklärliche Verschiedenheiten. Die ausgeprägtesten Bilder von Kolloidproduk-

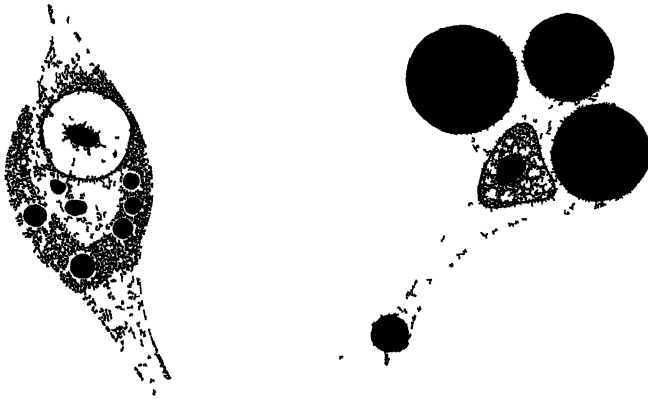


Fig. 9. Zwei Drüsen-Nervenzellen aus dem Nucleus supraopticus des Eichhornchens (*Sciurus vulgaris*). (Nach E. Scharrer, 1934 b.)

tion wurden beim Eichhornchen (*Sciurus vulgaris*) gefunden (Fig. 9). Nur wenige, dafür aber um so reicher mit Kolloid beladene Zellen weist der Kapuzineraffe auf. Im Verhältnis zu den Submammaliern sind aber die positiven Befunde bei den Säugern im Durchschnitt nicht so eindrucksvoll.

(7) HOMO

Die im Vorausgehenden geschilderten Befunde bei den Tieren boten die Grundlage für eine neue Erklärung der bisher schon mehrmals beschriebenen und vielfach als pathologisch betrachteten eigenartigen Zellbilder im Nucleus supraopticus und Nucleus paraventricularis des Menschen (E. Scharrer, 1933 a, Fig. 10). Die Erklärung der Struktureigentümlichkeiten dieser Zellen als zusammenhängend mit ihrer sekretorischen Tätigkeit konnte erstmals in einem Falle von Suicid eines jugendlichen, gesunden Menschen als richtig bestätigt werden (Scharrer & Gaupp, 1933). In diesem Falle konnten wie bei den Tieren Mehrkernigkeit der Zellen, peri- und endozelluläre Kapillaren, amitotische Kernteilungen und eingedellte Kerne im Bereich der Nucleus supraopticus und paraventricularis gefunden werden. Beweisend war aber die Feststellung von Kolloidtropfen im Zellplasma in der gleichen Form wie sie so häufig bei den verschiedensten Tiergattungen bereits gefunden worden waren. Dieser erste Befund beim Menschen wurde durch eine Reihe von in rascher Folge erscheinenden Arbeiten voll bestätigt. Gaupp (1934, 1935), Divry (1934), Roussy & Mosinger (1934), Gaupp & Scharrer (1935) und Peters (1935 a, b) berichteten über die gleichen Beobachtungen. Im ganzen sind bis

jetzt gegen 200 Fälle vom Menschen untersucht worden mit dem Ergebnis, dass der Befund von Kolloid im Zwischenhirn des Menschen ein ganz und gar unberechen-

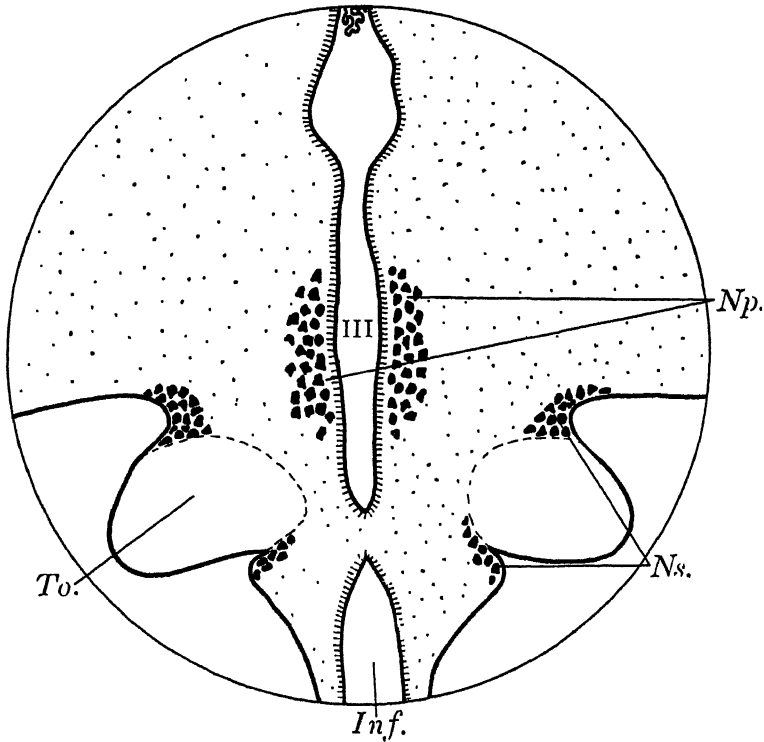


Fig. 10. Querschnitt durch das Zwischenhirn des Menschen. III=Dritter Ventrikel. *Inf.*=Infundibulum. *Np.*=Nucleus paraventricularis. *Ns.*=Nucleus supraopticus. *To.*=Tractus opticus. (Mit Benutzung einer Abbildung von Gagel, 1928.)

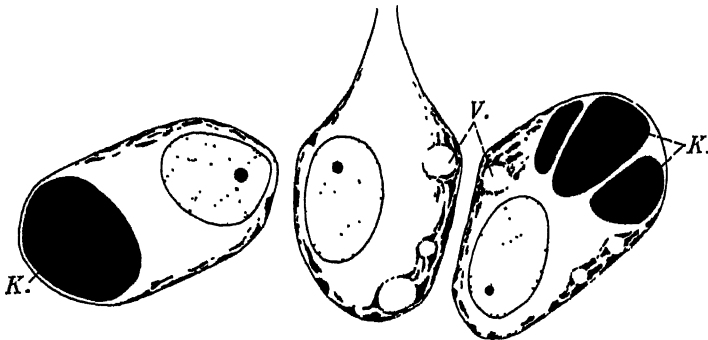


Fig. 11. Zellen aus dem Nucleus supraopticus des Menschen. *K.*=Kolloid. *V.*=Vakuolen. (Orig.)

bar verschiedener sein kann. Es wurden Fälle beobachtet, in denen beinahe jede Zelle einen oder mehrere Tropfen enthielt (Fig. 11), während in anderen leere Vakuolen vorherrschten oder schliesslich die Zellen überhaupt kein Anzeichen

einer sekretorischen Tätigkeit erkennen liessen. Divry sowohl als auch Gaupp und Peters haben eingehende Betrachtungen über einen möglichen Zusammenhang zwischen Erkrankungen der verschiedensten Art und dem Zustand der Zwischenhirndrüse angestellt. Es ergab sich keine sichere Beziehung zu Geistes- und Nervenkrankheiten und den bis jetzt untersuchten innersekretorischen Störungen. Auch das Alter ist beim Menschen für den Befund nicht ausschlaggebend. Die Sekretion scheint zwar im 4. bis 8. Lebensjahrzehnt ausgeprägter zu sein, fehlt aber nicht bei jugendlichen Individuen und kann auch in Fällen hohen Alters vermisst werden. Deutliche Unterschiede zwischen dem männlichen und dem weiblichen Geschlecht sind nicht vorhanden. Auch jahreszeitliche Schwankungen der Sekretion bestehen nicht. Im ganzen ergibt sich also eine weitgehende Übereinstimmung der Verhältnisse beim Menschen mit denen in den homologen Zwischenhirnkernen bei den Tieren.

IV. ÜBER DEN SEKRETIONSABLAUF IN DEN DRÜSEN-NERVENZELLEN

Gegen die hier zusammenfassend dargestellten Beobachtungen von sezernierenden Ganglienzellen im Zentralnervensystem der Wirbeltiere wurde eine Reihe von Einwänden erhoben. So lehnt Collin (1934) die Deutung der beschriebenen Zellbilder als verschiedene Stadien sekretorischer Vorgänge ab. Er führt in erster Linie ins Feld, dass es sich nicht um eine Sekretion von Kolloid durch die Nervenzellen handelt, sondern dass eine Aufnahme und Speicherung von Hypophysenkolloid vorliegt. Gegen eine Verallgemeinerung dieser Anschauung spricht nach unserer Meinung die Tatsache, dass Drüsen-Nervenzellen auch bei Wirbellosen vorkommen, die keine Hypophyse besitzen. Im speziellen Fall der Wirbeltiere, deren Zwischenhirndrüse den Ausgangspunkt unserer Untersuchungen bildete, zeigen die Rochen die Unhaltbarkeit der Collinschen Einwände. Während hier (z. B. bei *Raja asterias*) im Hypophysenzwischenlappen eine ausserordentlich lebhaft Kolloidproduktion stattfindet und Hypophysenkolloid also reichlich zur Verfügung steht, das in den wohl entwickelten Nucleus praeopticus auf den von Collin angegebenen Wegen einwandern könnte, weisen die Zellen des Nucleus praeopticus bei *Raja* doch nur sehr wenig, bei anderen Selachiern gar kein Kolloid auf. Dagegen wissen wir aus den Untersuchungen von Dahlgren (1914) und Speidel (1919), dass im Rückenmark von *Raja* und zwar im Schwanzteil grosse Drüsen-Nervenzellen mit reichlicher Kolloidproduktion in beträchtlicher Anzahl vorkommen. Es ist nicht wahrscheinlich, dass das Hypophysenkolloid in diesem Falle durch den grösseren Teil des Gehirns und durch das ganze Rückenmark bis zum Schwanzende wandert. So haben sich von der sekretorischen Natur der im Zwischenhirn der Wirbeltiere und des Menschen beobachteten Zellbilder inzwischen auch andere Untersucher überzeugt und unser Streit mit der Collinschen Schule darf als beendet angesehen werden.

Wenn damit auch die grundsätzliche Frage, ob Nervenzellen sekretorisch tätig sein können, in positivem Sinne beantwortet ist, so ist der Ablauf der Sekretion im einzelnen uns doch noch wenig bekannt. Wir wissen nur, dass die Kolloidtropfen

aus kleinen Granula hervorgehen, die man besonders schön bei der Kröte in Eisenhämatoxylinpräparaten beobachten kann. Über die Zytologie der Sekretbildung, etwa über die Frage der Beteiligung des Golgiapparates, liegen noch keine Untersuchungen vor. Sicher spielt der Kern bei der Sekretbildung eine grosse Rolle. Im Nucleus praeropticus von *Galeichthys feliceps* (vgl. S. 200) und im Nucleus lateralis tuberis von *Tinca vulgaris* (vgl. S. 201) kann die Bildung des Sekretes im Kern direkt beobachtet werden. Auch in der Oberflächenvergrößerung der Kerne sezernierender Zellen in Gestalt von fingerförmigen Ausläufern, abgeschnürten Kernsegmenten u.s.w. äussert sich die Beteiligung des Zellkerns. Am ausgeprägtesten sind solche Kernformen im Bereich des Nucleus lateralis tuberis der Fische, wo sie an die bekannten Kerngestalten mancher Drüsenzellen (Spinnrüden, Kittdrüsen u.s.w.) erinnern. Auch der Kern des Nervus terminalis, die Mittelhirngruppe und die Speidelsche Zellgruppe sind bei den Fischen durch vielgestaltige Kernformen ausgezeichnet.

Was das weitere Schicksal der reifen Kolloidtropfen anbelangt, so werden sie in der Mehrzahl der Fälle offenbar aus der Zelle ausgestossen und liegen dann zwischen den Zellen um hier einer allmählichen Auflösung zu verfallen. Bei der Ausstossung des Sekretes kann die Zelle selbst entweder völlig intakt bleiben (*Bufo*) oder es kommt zur Bildung grosser leerer Vakuolen, die nur noch einen Rest des Plasmas und den an die Wand gedrückten pyknotischen Kern enthalten (*Cristiceps*). Der Abtransport des Sekretes kann auf verschiedenen Wegen erfolgen. Manche Bilder sprechen dafür, dass das Kolloid auf dem Blutweg abtransportiert wird. Diese Form echter innerer Sekretion hält neuerdings Oliveira E. Silva (1935 a, b) beim Hunde für gegeben. Bei der Kröte kann man die Ausstossung von Sekretballen in den Ventrikel, im Besonderen in den Recessus praeropticus deutlich beobachten. Das ausgestossene Sekret ist wesentlich schlechter färbbar und wird offenbar im Liquor aufgelöst. Bei den Fischen und zwar bei der Schleie (*Tinca vulgaris*) nimmt das praeroptische Kolloid einen anderen, vom Ventrikel weggerichteten Weg. Es wandert entlang den Neuriten der Zellen des Nucleus praeropticus, die bei den Fischen den Tractus praethalamo-cinereus (Kappers, 1906; Röthig, 1911) bilden. Bei dieser Wanderung entlang dem genannten Faserbündel werden die Tropfen offenbar verbraucht, denn sie werden, je weiter man auf Schnittserien den Tractus praethalamo-cinereus von frontal nach kaudal verfolgt, immer kleiner, geringer an Zahl und schlechter färbbar. Neben der ungleichen Entwicklung der Zwischenhirndrüse bei den Wirbeltieren zeigt auch die verschiedene Art des Abtransportes des Sekretes bei Fischen und Amphibien, dass wir verschiedenartige Aufgaben der Zwischenhirnsekretion erwarten dürfen.

Der lebhafte Stoffwechsel, der den Drüsen-Nervenzellen sicherlich zukommt, drückt sich auch in der alle anderen Nervenzellen weit übertreffenden Blutversorgung aus. Die grossen Drüsen-Nervenzellen im Zwischenhirn der Säuger und des Menschen und die mächtigen Zellelemente im terminalen Rückenmarksabschnitt der Rochen sind von peri- und endozellulären Kapillaren versorgt (Collin, 1931; Scharrer & Gaupp, 1933) und die betreffenden Zellgebiete gehören zu den kapillarreichsten Bezirken des Gehirns überhaupt.

Bei den Wirbellosen spielen Gefäßversorgung und Kernformen nicht die gleiche Rolle für die besondere Auszeichnung der sekretorisch tätigen Nervenzellen; hier ist es der Befund von Sekrettropfen im Innern der Zellen, auf Grund dessen sie als Drüsen-Nervenzellen angesprochen werden.

V. ÜBER DIE HISTOCHEMISCHEN EIGENSCHAFTEN DES NEUROKOLLOIDS

Bezüglich der histochemischen Eigenschaften der im Vorausgehenden beschriebenen Sekrete ist zunächst zu betonen, dass es sich bei all den untersuchten Tieren und beim Menschen nicht um Fettsubstanzen handelt, da sie bei der Paraffin- bzw. Celloidin-einbettung nicht herausgelöst werden und auch färberische Reaktionen, die den Fetten eigen sind, nicht geben. In gleicher Weise bei Wirbellosen und bei Wirbeltieren färben sich die Neurosekrete mit dem Van Giesonschen Gemisch rot oder gelb, mit Heidenhains Eisenhämatoxylin schwarz. Fast stets finden wir das Kolloid im Zwischenhirn in Form regelmässig runder Tropfen, nicht als unregelmässig gestaltete Klumpen, wie das beim Hypophysenkolloid der Fall zu sein pflegt. Nicht selten auch können wir bei Vertretern von Wirbellosen wie von Wirbeltieren eine konzentrische Schichtung der einzelnen Tropfen in Gestalt verschieden gefärbter Ringe beobachten. In manchen Fällen enthalten die Kolloidtropfen in ihrem Inneren stark lichtbrechende Kriställchen.

Ähnlich charakterisiert Speidel (1919, 1922) die Sekrete der von ihm gefundenen Drüsen-Nervenzellen im Rückenmark der Rochen und Knochenfische. Diese Granula geben nicht die färberischen Reaktionen der Fette und Lipide, sind nicht in Wasser und Alkohol löslich wie die Zymogenkörner, geben aber eine positive Reaktion mit Millon, was ihren Eiweisscharakter dartut. Die Granula nehmen bei elektrischer Reizung des Rückenmarks oder bei Injektion von 0.1 %-iger Pilocarpinlösung in den Rückenmarkskanal an Zahl zu. Des weiteren konnte gezeigt werden, dass die Zellen nicht zum chromaffinen System gehören und nicht Adrenalin produzieren.

Was die besonderen färberischen Reaktionen des Zwischenhirnkolloids bei den Fischen anbelangt, so konnte hier festgestellt werden, dass sich das Kolloid ausser der schon genannten Färbung mit Van Gieson und mit Eisenhämatoxylin auch stark mit Eosin bzw. mit Orange G färben lässt. Bei der Azanfärbung nach Heidenhain erscheint das Kolloid rot und bei der Färbung nach Dominici erhält man es dunkelblau ebenso wie das Hypophysenkolloid. Bei der Nisslschen Färbung mit Toluidinblau oder Thionin lassen sich beide Kolloidarten nicht darstellen bzw. nur ganz schwach bläulich anfärben. Die Kolloideinschlüsse geben bei den Fischen keine Jodreaktion und lassen sich auch nicht mit Bestschem Karmin färben. Es kann sich also nicht um eine Art von Corpora amylacea handeln. Die Rotfärbung mit Van Gieson und die Violettfärbung mit Weigerts Methode der Fibrindarstellung lassen die sezernierenden Nervenzellen, wenn auch nur in ihrer äusseren Erscheinung, den schon genannten Russelschen Körperchen (kolloide Plasmazellen, Maulbeerzellen) vergleichbar erscheinen.

Bei der Kröte (*Bufo*) wurde das Sekret auch in unfixiertem Zustand untersucht. Folgende Methode erwies sich dabei als geeignet. Ein Tier wird dekapitiert, der Schädel von dorsal eröffnet und das Gehirn herausgenommen. Dieses wird in einem Schälchen mit Ringer-Lösung abgespült und auf einem Stück angefeuchteten Filtrierpapier mit der ventralen Seite nach oben auf dem Tisch des Gefriermikrotoms leicht angefroren. Mit einem Starmesser schneidet man, sobald das Gehirn etwas fest (nicht hart gefroren) geworden ist, den Bezirk des Nucleus praeropticus heraus, bringt ihn mit einem kleinen Tropfen Wasser auf einen Objektträger und zerdrückt ihn mit dem Deckglas zu einer dünnen Schicht. Bei genügender Abblendung sieht man auch im ungefärbten, frischen Zustand das Sekret in der streifenförmigen Anordnung des grosszelligen Anteils des Nucleus praeropticus.

Das frische, ungefärbte Sekret, das in der angegebenen Weise zur Anschauung gebracht werden kann, hat einen ganz leichten Stich ins Grünliche, wenigstens gegenüber dem unfixierten Nervengewebe in der Umgebung. Wird das Präparat nicht allzu stark gequetscht, dann behalten die kleineren Tropfen vielfach ihre traubenförmige Anordnung in den Zellen bei, wie man sie auch im gefärbten Celloidinschnitt konstatieren kann. Die grossen Tropfen sind von regelmässig runder Gestalt. Man findet alle die Formen, die im fixierten Schnittbild erscheinen, auch in einem derartigen frischen Quetschpräparat. Die Schnittbilder sind also nicht als künstliche Fixierungsprodukte zu betrachten. Das frische Sekret ist zähflüssig. Durch starken Deckglasdruck und infolge der Beugung durch das umgebende Nervengewebe ziehen sich die grossen Tropfen vielfach zu länglichen Gebilden aus. Auch die früher beschriebenen kleinen Vakuolen und Hohlräume innerhalb der Kolloidmasse fehlen im frischen Präparat nicht und sind also nicht durch Schrumpfung bei der Fixierung entstanden. Manche Tropfen lassen deutlich in ihrem Inneren einen kleinen, zentral liegenden Haufen kleiner, stark lichtbrechender Körperchen erkennen, wie sie in gleicher Weise bisweilen im fixierten Präparat beobachtet werden können. Entsprechende Befunde wurden bei den Fischen erhoben (Scharer, 1932 a). Das Kolloid der Kröten färbt sich im fixierten Zustand besonders distinkt mit Säurefuchsin. Es erschien nun interessant, ob das auch für das frische, nicht fixierte Kolloid gilt. Tatsächlich färbt sich dieses schnell und ausgiebig mit Säurefuchsin und hält den Farbstoff fest, wenn das unfixierte nervöse Gewebe durch Auswaschen mit Ringer-Lösung entfärbt wird. Schliesslich wurde auch lebenden Tieren Säurefuchsin injiziert und die Gehirne wurden nach verschiedenen Zeiträumen (3 Stunden, 2 Tage) untersucht. Es trat auf diese Weise keine Färbung des Sekretes ein.

Eingehende Untersuchungen über die Histochemie des Zwischenhirnkolloids beim Menschen hat Divry (1934) angestellt. Was die physikalischen Eigenschaften des Kolloids anbelangt, so bemerkte Divry ein starkes Lichtbrechungsvermögen des Kolloids. Das Zwischenhirnkolloid des Menschen ist weiterhin isotrop und bleibt es auch nach Behandlung mit Lugolscher Lösung oder mit Kongorot. Diese negative Eigenschaft gibt nach Divry die Möglichkeit das Kolloid von Amyloid deutlich zu unterscheiden. Die färberischen Eigenschaften des menschlichen Zwischenhirnkolloids sind die gleichen wie wir sie für die niederen Wirbeltiere

gefunden haben. Mit Nilblau, Sudan III, Scharlachrot und Neutralrot ist das Zwischenhirnkolloid nicht färbbar. Es handelt sich also nicht um Fettsubstanzen. Die Jodschwefelsäure-Reaktion ist negativ und auch sonst weist das Zwischenhirnkolloid des Menschen keine Verwandtschaft mit Glykogen, Amyloid und Corpora amylacea auf. Die aufgezählten Eigenschaften hat das Zwischenhirnkolloid nach Divry mit dem Kolloid der Hypophyse und der Schilddrüse gemein. Mehr lässt sich aber auf Grund des bisher vorliegenden Materials über den Chemismus der Zwischenhirnsekretion nicht aussagen.

VI. ÜBER DIE PHYSIOLOGISCHE BEDEUTUNG DER NEUROSEKRETION

Die bisherigen Untersuchungen waren histologischer Art und sollten zunächst nur die Verbreitung der Drüsen-Nervenzellen und der neurosekretorischen Organe bei den Tieren und beim Menschen klarstellen. Natürlich wurden von Anfang an Überlegungen über die mögliche physiologische Bedeutung der neurosekretorischen Vorgänge daran geknüpft und experimentell geprüft. Zunächst wurden Hoffnungen auf die neueren Untersuchungen über die chemische Übertragung nervöser Erregungen und die Lehre von den neurohumoralen Vorgängen gesetzt. Aber die neuesten Ergebnisse der dahin gerichteten Untersuchungen, wie sie Bacq (1935) zusammenfasste, lassen erkennen, dass die von uns beschriebenen neurosekretorischen Vorgänge mit der chemischen Übertragung der nervösen Erregung offenbar nichts zu tun haben. Auch die Erregungstoffe Steinachs (1929) und Haberlandts (1929, 1930) sowie die Schlaf- und Krampfstoffe Krolls (1933 *a, b*) werden überall im Gehirn gebildet und sind nicht an bestimmte Bildungsstätten innerhalb des Zentralnervensystems gebunden. Die Bildung von Stoffen wie Adrenalin und Acetylcholin, die ausser im Bereich der peripheren autonomen Nervenendigungen auch beim Übergang der Erregung von einem Neuron auf ein anderes eine Rolle spielen (Kibjiakow, 1933), erfolgt nicht so, dass histologisch nachweisbare Kolloidmassen auftreten. Schliesslich konnte Bacq (1935, S. 173) aus dem Gehirn von *Octopus* besonders grosse Mengen von Acetylcholin gewinnen, während wir gerade bei *Octopus* mit histologischen Methoden keinerlei Anzeichen einer sekretorischen Tätigkeit der Nervenzellen feststellen konnten.

Betrachtungen über die physiologische Bedeutung der Zwischenhirnsekretion der Wirbeltiere knüpften sich ganz von selbst zunächst an die Feststellung der Verbreitung der beobachteten Erscheinungen. Es ergab sich aber nicht der geringste Anhaltspunkt, der hier hätte weiterführen können. Nahe verwandte Amphibienarten ähnlicher Lebensweise zeigten grosse Unterschiede, während andererseits bestimmte Landtiere, marine Fische und Amphibien untereinander sehr übereinstimmende Bilder bieten konnten. Die Methode des Vergleichens von Tieren verschiedener Lebensweise, die sonst in der vergleichenden Neurobiologie eine so grosse Bedeutung hat, lässt hier völlig im Stich. Auch die geographische Herkunft der Tiere gleicher Art spielt keine Rolle. Wir untersuchten Vertreter von *Bufo vulgaris* aus verschiedenen Gegenden Deutschlands, aus der Umgebung Wiens und Neapels. Es waren keine wesentlichen Unterschiede zu finden. Ge-

schlecht, Alter und Jahreszeit spielen keine solche Rolle, dass daraus etwas auf die Bedeutung der Sekretion geschlossen werden könnte. (Bezüglich des Jahreszyklus des Nucleus lateralis tuberis der Fische und seines Zusammenhanges mit dem Funktionszyklus der Hypophyse wurde schon auf S. 201 das Wichtigste bemerkt.) Nur einige, freilich noch wenig eindeutige Befunde liegen vor:

(1) Bei den Bienen hat Weyer (1935) einen Zusammenhang mit dem Alter der Tiere festgestellt. Junge und alte Tiere zeigen nach Weyer wenig oder kein Sekret in ihrer "Gehirndrüse", während Trachtbienen mittleren Alters am meisten aufweisen. Ebenso ist die Sekretion bei Drohnen und bei der Bienenkönigin unbedeutender als bei der Arbeiterin. Die Stärke der Sekretion hängt also bei der Biene mit ihrer Aktivität zusammen.

(2) Bei den Fischen haben Untersuchungen an der Schleie (*Tinca vulgaris*) gezeigt, dass zu jeder Jahreszeit viel, wenig oder kein Kolloid im Nucleus praeropticus vorhanden sein kann. Das Alter spielt dabei insoweit eine Rolle, als junge, noch nicht geschlechtsreife Elritzen (*Phoxinus phoxinus*) noch kein Kolloid enthalten. Zwischen ♂ und ♀ bestehen keine Unterschiede und die Laichzeit hat keinen Einfluss auf die Menge des Kolloids im Nucleus praeropticus. Anders liegen die Verhältnisse im Nucleus lateralis tuberis, aber hier spielen die jahreszeitlichen Einflüsse der Hypophyse herein und wir dürfen die deutlichen Unterschiede, die der Nucleus lateralis tuberis im Sommer und im Winter bietet, nicht ohne Vorbehalt als Sekretionsrhythmus der Zellen des Nucleus lateralis tuberis werten. Die früher geäußerte Hypothese, dass die Zwischenhirnsekretion bei den Fischen etwas mit dem Farbwechsel zu tun hat (Scharrer, 1932 a), kann in dieser Form nicht aufrecht erhalten werden. Wir brauchen die zahlreichen Versuche auf irgend einem Wege an die Klärung der Verhältnisse bei den Fischen heranzukommen nicht im einzelnen zu schildern; hier interessiert nur das Ergebnis und dieses war bis jetzt durchaus negativ.

(3) Nicht viel anders steht es mit den Amphibien. Hier schien durch die Versuche von Houssay & Giusti (1930) die Rolle des Hypothalamus für die Häutung erwiesen, aber es hat sich herausgestellt, dass der Hypophyse die Hauptbedeutung zukommt. Die Wirkung einer Hypothalamusläsion macht sich durch eine Schädigung der Hypophysenfunktion und damit sekundär durch Häutungsstörungen bemerkbar. Es lässt sich zeigen, dass die Einpflanzung von kolloidreichem Zwischenhirn keine Wirkung auf die Häutung hypophysektomierter Kröten hat, während Hypophysenimplantation in wenigen Stunden Häutung auszulösen vermag (Scharrer & Gaupp, 1935). Wir können also nur so viel sagen, dass das die Häutung der Kröten regulierende Hormon von der Zwischenhirndrüse nicht gebildet wird.

(4) Bei den Säugern konnte keine Beziehung zwischen dem Kolloidgehalt des Hypothalamus und der Jahreszeit festgestellt werden. Auch beim Menschen bestehen keine Zusammenhänge zwischen Alter, Jahreszeit, Geschlecht, Art der Erkrankung und der Menge des Zwischenhirnkolloids. Es wurde bereits in früheren Mitteilungen (Gaupp & Scharrer, 1935) auf die Möglichkeiten vor allem in Hinblick auf die Regulation des Wasserhaushalts hingewiesen. Wir wollen die

Betrachtungen darüber nicht wiederholen, da irgendwelche Beweise für ihre Richtigkeit inzwischen nicht erbracht worden sind.

Zusammenfassend möchten wir bezüglich der Neurosekretion im Allgemeinen und der Zwischenhirnsekretion im Besonderen feststellen, dass in keinem Falle bei Wirbellosen und Wirbeltieren etwas bestimmtes über die funktionelle Bedeutung der neurosekretorischen Vorgänge bis heute bekannt ist. Indessen erscheint die histologische Basis nun breit genug, dass man an die physiologische Untersuchung mit Aussicht auf Erfolg gehen kann.

VII. ZUSAMMENFASSUNG

Wir stellen hier die "Drüsen-Nervenzelle" als einen neuen Zelltypus zur Diskussion. Wir verstehen darunter Nervenzellen, die mehr oder weniger das Bild sekretorisch tätiger Elemente bieten und die innerhalb des Zentralnervensystems in der Regel wohl abgrenzbare "Organe" bilden, wie z. B. die Zwischenhirndrüse der Wirbeltiere. Im einzelnen kann auf Grund des bis jetzt vorliegenden Materials bezüglich dieser Zellen folgendes festgestellt werden:

(1) Die Drüsen-Nervenzellen sind weit verbreitet. Unter den Wirbellosen wurden sie bei Anneliden, Mollusken, Crustaceen und Insekten gefunden. In ihrem feineren Aufbau konnte eine vollkommene Übereinstimmung zwischen den Drüsen-Nervenzellen bei den Bienen und bei den Opisthobranchiern festgestellt werden. Bei den Wirbeltieren finden sich lebhaft sekretorisch tätige Zellen in erster Linie im Zwischenhirn und zwar bei Selachiern, Knochenfischen, Amphibien, Reptilien, Säugern und beim Menschen. Bei Knochenfischen wurden Drüsen-Nervenzellen weiterhin im Kern des Nervus terminalis, im Haubengebiet des Mittelhirns und bei einzelnen Arten im Schwanzabschnitt des Rückenmarks festgestellt. Bei Selachiern (*Raja*) sind diese letzteren besonders gut entwickelt. Die Drüsen-Nervenzellen kommen also bei Wirbellosen und Wirbeltieren in verschiedenen Abschnitten des zentralen Nervensystems vor.

(2) Es handelt sich um echte Nervenzellen oder um von Nervenzellen abzuleitende Elemente. Die grossen Drüsenzellen im terminalen Rückenmarksabschnitt der Rochen entwickeln sich aus den gleichen Neuroblasten wie die Vorderhornzellen des Rückenmarks. Bei Knochenfischen können ferner bei vergleichender Untersuchung einer grösseren Anzahl von Arten alle Übergänge beobachtet werden von typischen Nervenzellen ohne histologisch nachweisbare Sekretproduktion (z. B. *Salmo fario*) über verschiedene Grade der Kolloidbildung und -speicherung (*Tinca vulgaris*, *Phoxinus laevis*, *Fundulus heteroclitus*) bis zur Umwandlung der Zellen in Drüsenzellen, die alle nervösen Charaktere vermissen lassen (*Cristiceps argentatus*, *Galeichthys feliceps*). Auch bei den Amphibien steht die nervöse Natur der Zellen ausser Frage, denn auch solche Zellen, die mit Sekretgranula aller Grössen angefüllt sind, lassen doch deutlich lange Ausläufer und Nisslschollen erkennen.

(3) Gemeinsam ist allen Drüsen-Nervenzellen der Wirbellosen und Wirbeltiere die Bildung und Abgabe von Granula und Kolloidtropfen. Diese treten teils im

Plasma selbst auf (*Aplysia*, *Pleurobranchaea*, *Bufo*), teils sind sie in Vakuolen eingeschlossen (*Raja*, *Cristiceps*). Ihre Färbbarkeit ist unspezifisch.

(4) Für die Mehrzahl der bisher beobachteten Drüsen-Nervenzellen ist ein ausgeprägter Kernpolymorphismus charakteristisch. So sind die Kerne der Drüsen-Nervenzellen im Rückenmark von *Raja*, im Nucleus lateralis tuberis von *Esox lucius* und *Tetrodon lagocephalus*, in der Mittelhirndrüse von *Phoxinus laevis* u.s.w. vielfältig gelappt und verzweigt, sodass auf dem Schnitt Bilder wie von polymorphkernigen Leukocyten oder vielkernigen Riesenzellen entstehen. Der lebhafte Stoffwechsel der sekretorisch tätigen Nervenzellen macht wohl eine grosse Kernoberfläche notwendig, die hier, wie auch sonst vielfach bei Drüsenzellen, durch eine gelappte und verzweigte Form des Zellkerns erreicht wird.

(5) Ebenfalls im Zusammenhang mit dem lebhaften Stoffaustausch der Drüsen-Nervenzellen steht ihr Verhalten zu den Gefässen. So ziehen jeweils vier bis fünf Kapillaren um eine der grossen Drüsen-Nervenzellen im Rückenmark von *Raja*; bisweilen ist eine Kapillare auch ganz vom Zelleib eingeschlossen. Solche perizelluläre und endozelluläre Kapillaren wurden auch in den sekretorisch tätigen Zwischenhirnkernen der Wirbeltiere beobachtet.

(6) Welche Stoffe in den durch histologische Untersuchung bis jetzt bekannt gewordenen intrazentralen Drüsengebieten gebildet werden und welche physiologische Bedeutung ihnen zukommt, ist noch unerforscht.

VIII. SUMMARY

In this article the gland-nerve cell is considered as a new type of cell. By the term "gland-nerve cell" is meant nerve cells having more or less the appearance of secretory cells. They may form well-delimited "organs" within the central nervous system, as, for example, the diencephalic gland of vertebrates. The following facts are known concerning these cells.

(1) Gland-nerve cells have a wide distribution. Among the invertebrates, they have been found in annelids, molluscs, crustaceans and insects. There is a close resemblance in detailed structure between the gland-nerve cells of bees and of opisthobranchs. In the vertebrates, active secretory cells are found chiefly in the diencephalon of selachians, teleosts, amphibia, reptiles, and mammals, including man. In the bony fishes, gland-nerve cells have also been found in the nucleus of the nervus terminalis, in the midbrain, and, in some genera, in the caudal region of the spinal cord. In selachians (*Raia*) they are specially well developed. Thus gland-nerve cells occur in various regions of the central nervous system both of invertebrates and vertebrates.

(2) Gland-nerve cells are either true nerve cells or are derivatives of nerve cells. The large gland cells in the terminal region of the spinal cord of the skate develop from the same neuroblasts as the cells in the anterior horn. In bony fishes, all stages can be found from typical nerve cells without histologically visible secretory products (*Salmo*), through cells with varying degrees of colloid formation and storage (*Tinca*, *Phoxinus*, *Fundulus*), to cells transformed into gland cells lacking any nervous character (*Cristiceps*, *Galeichthys*). The nervous nature of such cells cannot be doubted in the amphibia, for even cells which are filled with secretory granules of all sizes have long processes and Nissl granules.

(3) All gland-nerve cells, both of invertebrates and vertebrates, produce granules and drops of colloid. In some cases these secretions appear in the cytoplasm itself (*Aplysia*,

Pleurobranchaea, *Bufo*), in other cases they are included in vacuoles (*Raia*, *Cristiceps*). Their staining properties are non-specific.

(4) A marked nuclear polymorphism is characteristic of most gland-nerve cells. Thus the nuclei of such cells in the spinal cord of *Raia*, in the nucleus lateralis tuberis of *Esox* and *Tetodon*, in the midbrain gland of *Phoxinus*, etc., are lobed and branched, so that the aspect of polymorphonuclear leucocytes or multinucleate giant cells is given in sections. Doubtless the active metabolism of secretory nerve cells requires a large nuclear surface, supplied here, as is often the case in gland cells, by the lobed and branched form of the nucleus.

(5) The relation of gland-nerve cells to blood vessels is likewise connected with their active metabolism. Thus four or five capillaries sometimes surround a large gland-nerve cell in the spinal cord of *Raia*, and a capillary may be enclosed by the cell body. Such pericellular and endocellular capillaries have also been observed in the secretory diencephalon nuclei of vertebrates.

(6) It is as yet unknown what substances are secreted by glandular regions of the central nervous system and what is their physiological role.

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ÜBER DIE SEKRETION UND RESORPTION VON GASEN IN DER FISCHSCHWIMMBLASE

VON JOACHIM FRHR. VON LEDEBUR

(Breslau)

(Received 16 March, 1936)

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I. EINLEITUNG

BEI der Analyse der Schwimmbblasengase vieler Fische, besonders von in grösserer Meerestiefe gefangenen Physoklisten, d. h. Fischen mit allseitig geschlossener Schwimmbblase, wurde von vielen Forschern ein z. T. erstaunlich hoher Sauerstoffgehalt beobachtet. Es wurden Werte des Sauerstoffprozentgehaltes gefunden, die weit über denen der atmosphärischen Luft lagen, und die, besonders auch bei Berücksichtigung des hohen Druckes, unter dem die Schwimmbblasengase stehen können, schon frühzeitig (Biot, 1807) zu der Annahme führten, dass die Ansammlung und die Zusammensetzung der Schwimmbblasengase nicht einfach physikalisch durch Diffusionsvorgänge erklärt werden können, sondern dass besondere Lebensvorgänge, Sekretionsprozesse, dabei eine Rolle spielen müssen. Auch für den Stickstoff wurden Beobachtungen bekannt (Hüfner, 1892), bei denen zur Erklärung einfache Diffusionsvorgänge nicht mehr ausreichten. Nachdem durch die Untersuchungen von A. & M. Krogh (1909-10) (s. a. Liljestrand, 1925) für die Gasaustauschvorgänge in der Lunge gezeigt werden konnte, dass hier Diffusionsvorgänge zur Erklärung ausreichen (s. dagegen die gegenteilige Ansicht von Haldane, 1922), ist die Schwimmbblase als das einzige bekannte Organ bei den Wirbeltieren übrig geblieben, in dem eine Gassekretion vorkommen soll. Wegen des allgemein-physiologischen Interesses, den ein solcher Vorgang einer Gassekretion besitzt, soll im folgenden über die bei dieser Ausscheidung von Gasen auftretenden Probleme und die zu ihrer Erklärung aufgestellten Theorien berichtet werden.

II. PHYSIKALISCHE VORBEMERKUNGEN

Die Zusammensetzung des in der Schwimmblase gefundenen Gasgemisches—Sauerstoff, Kohlensäure, Stickstoff und Edelgase wurden nachgewiesen—ist bei den verschiedenen Fischarten eine sehr wechselnde. Besonders der Anteil des Sauerstoffs ist sehr schwankend, Sauerstoffwerte zwischen 0·5 und über 90 % wurden beobachtet; der Kohlensäuregehalt kann 0–10 %, im Mittel 2–4 % betragen, der Rest sind Stickstoff und Edelgase (s. Tabelle I). Aber auch zwischen den Tieren derselben Fischart sind mehr oder weniger starke Unterschiede des Gehaltes an einzelnen Gasen in der Schwimmblase vorhanden, die allerdings bei

Tabelle I. Zusammensetzung der Schwimmblasengase einiger Fischarten in Prozent

Fischart	Blasengase normal		Blasengase nach Punktion (P.) oder nach Druckerhöhung (Dr.)			Autor
	O ₂	CO ₂	O ₂	CO ₂		
<i>Anguilla vulg.</i>	32·4	—	63·0	—	(Dr.)	Popta, 1910
<i>Box salpa</i>	13·3	1·1	33·8	4·3	(P.)	Jacobs, 1934
<i>Cantharus lineat.</i>	37·1	0·5	82·1	3·3	(P.)	v. Ledebur (unveröf.)
<i>Conger vulg.</i> (Tiefe)	87·7	0·4	—	—	—	Richard, 1895
<i>Coregonus acronius</i> (Tiefe)	1·5	0·7	—	—	—	Hüfner, 1892
<i>Crenilabrus pavo</i>	23·4	1·7	45·2	1·8	(P.)	Jacobs, 1934
„	31·2	1·0	84·5	3·2	(P.)	v. Ledebur (unveröf.)
<i>Cyprinus carpio</i>	5·8	3·6	6·7	3·2	(P.)	Hall, 1924
<i>Esox lucius</i>	25·6	1·8	—	—	—	Popta, 1910
„	19·8–44	3·9–8·9	24·2–75	5·5–15·8	(P.)	Jacobs, 1934
<i>Gadus callarias</i>	15·0	—	79–84	—	(P.)	Bohr, 1894
<i>Gadus morrhua</i>	19·0	0·5	48·0	2·8	(P.)	Jacobs, 1934
„	7–44·8	0·2–1·1	—	—	—	„
<i>Labrus festivus</i>	19·6	0·7	43·7	1·5	(P.)	„
<i>Labrus maculatus</i>	22·4	—	45·0	—	(Dr.)	A. Moreau, 1877
<i>Labrus turdus</i>	8·6	0·6	24·5	1·0	(P.)	Jacobs, 1934
<i>Labrus variegatus</i>	19·0	—	57–85	—	(P.)	A. Moreau, 1877
<i>Leuciscus dobula</i>	12·2	—	23·9	—	(P.)	Hüfner, 1892
<i>Lota vulgaris</i> (Tiefe)	64·8	—	—	—	—	„
<i>Mugil cephalus</i>	16·1	—	30·0	—	(Dr.)	A. Moreau, 1877
<i>Muraena conger</i>	30·0	—	62–87	—	(P.)	„
<i>Pagellus erythrinus</i>	53·4	3·9	86·4	3·1	(P.)	v. Ledebur (unveröf.)
<i>Perca fluviatilis</i>	19–25	—	40–65	—	(P.)	A. Moreau, 1877
„	12·4	1·4	22·0	10·6	(P.)	Jacobs, 1934
<i>Phoxinus laevis</i>	8·7	2·0	42·6	5·3	(P.)	„
<i>Salmo irideus</i>	3·7	0·8	—	—	—	„
<i>Sargus Rondeletii</i>	26·0	0·5	59·4	2·6	(P.)	„
<i>Serranus cabrilla</i> (Oberfläche)	18·0	0·9	—	—	—	v. Ledebur (unveröf.)
(Tiefe)	72·0	3·5	—	—	—	„
<i>Serranus cabrilla</i>	38·6	0·7	72·6	0·6	(P.)	„
<i>Smaris alcedo</i>	51·0	0·5	83·0	1·5	(P.)	„
<i>Tinca vulgaris</i>	4·1	5·8	—	—	—	Jacobs, 1934
„	1·9	2·6	22·9	13·0	(P.)	„
„	6–8	—	60·0	—	(P.)	A. Moreau, 1877
<i>Trigla cucullus</i> (Oberfläche)	27·3	—	—	—	—	Delaroche, 1809
(Tiefe)	70	—	—	—	—	„
<i>Trigla</i>	16	—	52	—	(Dr.)	A. Moreau, 1877

Fischen, welche längere Zeit unter möglichst gleichen Bedingungen gehalten wurden, in der Regel nur wenige Prozente betragen, die aber exakte Versuche über Änderungen in der Blasengaszusammensetzung an verschiedenen Fischen doch nicht zulassen (s. unten).

Um nun überhaupt die Frage entscheiden zu können, wie diese verschiedenen Gase in die Schwimmbase hereinkommen können, und wie weit eine Gasdiffusion zur Erklärung der Zusammensetzung und des Gasdruckes der Schwimmbasengase ausreicht oder nicht, müssen zuerst die physikalischen Verhältnisse klargestellt sein, unter denen sich die Schwimmbasengase, die im Blut und im umgebenden Wasser gelösten Gase befinden, und welchen Einfluss besonders der Druck der Wassersäule in grösserer Meerestiefe auf diese Gase ausübt. Dabei sollen hier wegen der besseren Übersicht vorerst nur die Verhältnisse bei den Physoklisten¹ betrachtet werden, bei denen eine Aufnahme atmosphärischer Luft durch Luftschnappen wegen der fehlenden Verbindung zwischen der Schwimmbase und dem Darmkanal sicher nicht möglich ist. Die Schwimmbasengase können also bei diesen Fischen nur auf dem Blutwege in die Base gelangt sein. Um zu erfahren, wie weit dabei Diffusionsprozesse eine Rolle spielen können, muss zuerst der Druck dieser verschiedenen Gase bekannt sein, da eine Gasdiffusion immer nur von Orten höheren Druckes zu Orten niedrigeren Druckes erfolgen kann.

Was zunächst den Gasdruck der einzelnen im Wasser gelösten Gase betrifft, so ist derselbe nur von der Zusammensetzung der umgebenden Luft abhängig. Er kann höchstens gleich dem Teildruck sein, den die einzelnen Gase in der atmosphärischen Luft besitzen, die mit dem Wasser an der Berührungsstelle zwischen Wasser und Luft im Diffusionsausgleich stehen. Die Höhe der Wassersäule, die Grösse des Wasserdruckes ist dabei ohne jeden Einfluss auf die Grösse des Gasdruckes der im Wasser gelösten Gase. D. h. auch in grosser Wassertiefe kann die Gasspannung der einzelnen im Wasser gelösten Gase höchstens so gross sein wie an der Oberfläche, also wie in der atmosphärischen Luft. Sie ist, wie die Untersuchung von Wasserproben aus grösserer Meerestiefe ergab, sogar in der Regel niedriger. Und ebenso wenig wird natürlich die Gasspannung des in den Kiemen mit dem umgebenden Wasser in Diffusionsausgleich stehenden Blutes von der Höhe des Wasserdruckes beeinflusst. Weder die aus grosser Meerestiefe heraufgeholtten Wasserproben noch das Blut von Fischen (v. Ledeboer, 1936), die aus grosser Tiefe an die Oberfläche gebracht wurden, schäumten an der Oberfläche bei dem Verschwinden des vorher auf ihnen ruhenden Wasserdruckes von vielen Atmosphären auf, ein Zeichen, dass die in ihnen gelöst vorhandenen Gase unter keinem höheren Druck als 1 Atmosphäre gestanden haben können. (Ein Freiwerden von Gasbläschen im Blut, analog der Caissonkrankheit, tritt aber natürlich auf, wenn Fische, die in Wasser, auf dem ein Gasdruck von mehreren Atmosphären lastet, gehalten worden sind, plötzlich in Wasser gebracht werden, das nur unter Atmosphärendruck steht (Moreau, 1876).)

¹ D. h. Fische mit allseitig geschlossener Schwimmbase, im Gegensatz zu den Physostomen, deren Schwimmbase durch einen Verbindungsgang, den Ductus pneumaticus, mit dem Darmkanal in Verbindung steht.

Ganz anders liegen die Verhältnisse jedoch bei den Blasengasen. Bei einer Ansammlung freien Gases unter Wasser wird der Druck dieser Gase direkt um den Druck der darüberstehenden Wassersäule erhöht; er wird infolgedessen immer grösser sein als der Druck der gelösten Gase. Durch einfache Diffusionsvorgänge kann daher niemals unter Wasser eine freie Gasblase gebildet werden. Eine Gasblase, ganz gleich welcher Zusammensetzung, ist unter Wasser aber auch nicht haltbar: infolge des entsprechend der Wassertiefe erhöhten Druckes der Gase findet durch verschiedene Diffusionsvorgänge eine allmähliche Resorption der Gase im Wasser statt.

Genau das gleiche gilt natürlich auch für die Ansammlung von Gasen innerhalb einer dünnen, für die Gase durchlässigen Membran. Bei der Umhüllung der Gasansammlung durch eine völlig gasundurchlässige Membran würden die einmal vorhandenen Gase ebenfalls unter einem, entsprechend der Wassersäulenhöhe erhöhtem Druck stehen, wobei ihr Volumen entsprechend verringert würde. Es würden jedoch auch in grösserer Tiefe keine Gase entweichen können. Es würden aber auch keine Gase in die Schwimmblase hereinkommen können, eine Erscheinung, die man jedoch an den Fischen gerade bei Volumabnahme der Blasengase (Punktion, Druckerhöhung durch grössere Wassertiefe usw. s. unten) beobachten kann; ganz abgesehen davon, dass das Gas ja erst einmal in diese undurchlässige Blase irgend wie hereingelangt sein muss.

Die Beobachtung von v. Ledebur (1928) (s. a. v. Baer, 1835, 1837), dass Jungfische auch von Physoklisten (Stichling usw.) kurz nach der Geburt an der Wasseroberfläche Luft schnappen und diese durch einen nur wenige Tage lang bestehenden Verbindungsgang zwischen Darm und Schwimmblase in das Blasenlumen bringen, kann hier nicht als Einwand angeführt werden. Die von den Jungfischen aufgenommenen Luftbläschen sind minimal klein und reichen gerade nur zur Füllung ihrer Schwimmblase aus. Wegen des sehr schnell sich wieder rückbildenden Luftganges kann eine weitere Aufnahme atmosphärischer Luft während des Wachstums nicht mehr stattfinden. Immerhin ist es interessant, dass jedenfalls bei den untersuchten Physoklisten der für eine physikalische Erklärung besonders schwierige Vorgang der erstmaligen Gasfüllung dadurch umgangen wird, dass atmosphärische Luft durch Luftschnappen in die Schwimmblase gebracht wird und gebracht werden muss. Verhindert man nämlich die Jungfische am Luftschnappen, bis der Luftgang obliteriert ist, dann kann die Schwimmblase dieser Fische niemals mehr mit Gas gefüllt werden, sie bleibt gasleer. (Die erwachsenen Fische, deren Gasdrüse schon einmal tätig gewesen war, besaßen dagegen die Fähigkeit, in eine künstlich vollständig mit Ringerlösung gefüllte Schwimmblase wieder Gase auszuschcheiden.)

Nur wenn die Gasblase eine starre, unnachgiebige Wandung besitzt, wird der Gasdruck durch den Wasserdruck nicht beeinflusst, der Druck der Wassersäule wird durch die starre Wandung abgehalten. Auch in beliebiger Wassertiefe würde eine derartige Gasblase, wenn sie einmal vorhanden ist, in ihrer ursprünglichen Grösse bestehen bleiben können, und durch Diffusionsprozesse würden bei permeabler Membran die Gase im Innern die Zusammensetzung annehmen, die dem Druck der einzelnen im Wasser gelösten Gase entspricht, also ungefähr wie in

der Atmosphäre. Abgesehen davon, dass die Zusammensetzung der Schwimmblasengase der Fische, besonders der Tiefseefische, wie oben erwähnt, stark von der der atmosphärischen Luft abweicht, ist die Fischschwimmbase nicht von einer derartig starren Membran umgeben. Sie liegt zwar dicht unter der Wirbelsäule und wird seitlich von der durch die Rippen gestützten seitlichen Bauchwand bedeckt, aber Beobachtungen und Versuche zeigen deutlich, dass die Blasengase unter einem von der Wassertiefe abhängigen Druck stehen. Es ist da einmal die Erscheinung der "Trommelsucht" zu erwähnen: aus grosser Meerestiefe plötzlich an die Wasseroberfläche gebrachte Fische treiben mit dem Rücken nach unten hilflos an dieser umher und zeigen einen stark aufgetriebenen Bauch, ein Herausquellen von Eingeweiden aus der Mundhöhle infolge einer durch die Druckverminderung sehr starken Ausdehnung der Blasengase, die meist sogar ein Platzen der Schwimmblase bewirkt. Diese Erscheinungen sind um so stärker, je grösser die Meerestiefe war, aus der die Fische heraufgeholt wurden. Aber auch durch Versuche konnte gezeigt werden, dass das Volumen der Fische durch Änderungen des auf ihnen lastenden Druckes beeinflusst werden kann, dass also eine Einwirkung des Aussendruckes auf die Gase der Schwimmblase möglich und vorhanden ist (Moreau, 1876). Die Druckerhöhung verursacht infolge Kompression der Blasengase eine Erhöhung des spezifischen Gewichtes der Fische, sie werden zu schwer und sinken zu Boden. Umgekehrt tritt bei Druckerniedrigung durch Ausdehnung der Blasengase eine Erniedrigung des spezifischen Gewichtes ein, die Fische werden zu leicht und werden an die Oberfläche getrieben. Und schliesslich soll nach den Versuchen von Hüfner (1892) bei *Leuciscus dobula* durch Entleerung der Schwimmblase (Punktion) in derselben kein Unterdruck entstehen, was bei starren Wänden der Fall sein müsste.

Wir können also zusammenfassen, dass der Gasdruck der Schwimmblasengase, die von einer nachgiebigen und sicher für Gase durchlässigen (s. unten) Membran umgeben sind, immer—und zwar umso mehr, in je grösserer Wassertiefe die Fische sich befinden—grösser als der des Blutes und der des Wassers ist, in dem die Fische leben. Hall (1924) konnte bei *Micropterus* direkt nachweisen, dass der Sauerstoffdruck der Blasengase dieses Fisches grösser als der des Blutes war, da Blut mit Blasengasen in Berührung gebracht noch Sauerstoff aus diesen aufnahm. Durch einfache Diffusionsvorgänge kann weder die Entstehung noch überhaupt das längere Bestehenbleiben der Gasansammlung in der Schwimmblase, weder die beobachtete Zunahme der Gase während des Wachstums der Fische, nach Volumverminderungen (Hinabtauchen in grössere Tiefen usw.), noch der z. T. beträchtlich über dem Prozentgehalt der atmosphärischen Luft liegende Sauerstoff- oder Stickstoffgehalt der Blasengase erklärt werden. Am deutlichsten werden wohl die hier herrschenden Verhältnisse durch ein des öfteren erwähntes Beispiel gekennzeichnet: Schlösing & Richard (1896) untersuchten die Zusammensetzung der Schwimmblasengase eines in einer Tiefe von 4500 Fuss gefangenen Fisches, die also unter einem Druck von 150 Atmosphären gestanden hatten, und fanden einen Gehalt von 85 % Sauerstoff, 12 % Stickstoff und Argon und 3 % Kohlensäure. Der Partialdruck des Sauerstoffs in der Schwimmblase dieses Fisches betrug

demnach 127 Atmosphären, der des Stickstoffs und Argons 18 Atmosphären und der der Kohlensäure 5 Atmosphären. Wenn wir bedenken, dass der Gasdruck des Sauerstoffs im Wasser (und damit auch im Blut) ungefähr $\frac{1}{5}$ Atmosphäre, und der des Stickstoffs $\frac{4}{5}$ Atmosphäre gross ist, wird es klar, wie wenig Diffusionsvorgänge hier zur Erklärung herangezogen werden können. Es wird vielmehr die Grösse der aktiven Zelltätigkeit deutlich, der es gelingt, Gase bei diesem hohen Druck in die Blase hereinzuschaffen.

Und zwar handelt es sich dabei, wie aus dem zitierten Beispiel deutlich wird, und besonders hervorgehoben werden muss, nicht nur um eine aktive Ausscheidung von Sauerstoff entgegen dem Druckgefälle, sondern auch der Stickstoff und die Kohlensäure müssen aktiv in die Blase ausgeschieden worden sein. Als besonders typisches Beispiel für eine derartige aktive Sekretion von Stickstoff ist hier noch der im Bodensee in einer Tiefe von 60–80 m. lebende Kilch (*Coregonius acronius*) zu erwähnen, dessen Schwimmblasengase nach Hübner (1892) in vielen Fällen einen Gehalt von weit über 90 % Stickstoff (bis zu 99 %) besaßen. Aber auch der Stickstoffgehalt von nicht in so grosser Tiefe lebenden Fischen ist häufig grösser, als dass er durch Diffusionsprozesse erklärbar wäre. Nach Winterstein (1921) würde in einer Wassertiefe von 10 m. der höchste durch Diffusion erklärbare Sauerstoffgehalt 10.5 % und der Stickstoffgehalt 39.5 %, in einer Tiefe von 1000 m. nur noch 0.21 % für den Sauerstoff und 0.79 % für den Stickstoff betragen können. Zur Erklärung der Zusammensetzung der Blasengase muss daher, worauf schon Hübner besonders hingewiesen hat, ausser einer Sauerstoffsekretion auch eine Stickstoffsekretion angenommen werden; von den aufgestellten Gassekretionstheorien (s. unten) muss verlangt werden, dass sie auf diese Tatsache Rücksicht nehmen.

III. BEDINGUNGEN DER GASAUSSCHIEDUNG UND GASRESORPTION

Wie oben schon erwähnt, besitzen die Fische, wie durch eine grosse Zahl von Versuchen sichergestellt ist, die Fähigkeit, unter besonderen Bedingungen Gase in die Schwimmblase auszuschieden. Durch A. Moreau (1877) konnte zuerst gezeigt werden, dass eine derartige Gasausscheidung erfolgt, wenn aus irgend einem Grunde eine Vergrösserung des spezifischen Gewichtes des Fisches, eine Volumabnahme der Blasengase eintritt. Sowohl die direkte Entleerung der Schwimmblase durch Punktion als auch die Verminderung des Blasengasvolumens durch Erhöhung des auf dem Fisch lastenden Druckes oder die Erhöhung des spezifischen Gewichtes durch Befestigung eines Bleigewichtes an dem Fisch hat die gleiche Wirkung: Ausscheidung von Gasen in die Schwimmblase bis zur Wiederherstellung des ursprünglichen spezifischen Gewichtes (Baglioni, 1908; Popta, 1910). Die nach der Erhöhung des spezifischen Gewichtes durch die verschiedenen Versuchsbedingungen am Boden der Aquarien liegenden, sich nur schwer vom Grunde erhebenden und sofort wieder zu Boden sinkenden Fische gewinnen nach einiger Zeit ihr ursprüngliches spezifisches Gewicht und damit ihre freie Beweglichkeit wieder. Auf die aus diesen Versuchsergebnissen abgeleitete Bedeutung der Schwimmblase als hydrostatisches Organ und als statisches Sinnesorgan soll hier nicht eingegangen

werden (Mangold, 1913; du Bois-Reymond, 1914; Fischer, 1930; s. a. Jacobs, 1935). Hier interessiert uns nur, dass eine Gasausscheidung in die Schwimmbase stattfindet nicht nur bei Druckerniedrigung der Blasengase (Punktion), sondern auch bei gleichbleibendem und sogar bei Erhöhung des Blaseninnendruckes. Besonders der letzte Fall zeigt wieder deutlich, dass Diffusionsvorgänge hier zur Erklärung nicht herangezogen werden können.

Verringerung des spezifischen Gewichtes, Injektion von Gasen in die Schwimmbase, Erniedrigung des auf den Fischen lastenden Wasserdruckes oder Anhängen eines mit Gas gefüllten Glasballons an den Fisch bewirkt eine Resorption von Gasen aus der Schwimmbase, und zwar solange, bis die immer wieder an die Wasseroberfläche getriebenen und nur mit Anstrengung in tiefere Regionen des Wassers gelangenden Fische (besonders deutlich bei den trommelsüchtigen Fischen) wieder leicht beweglich umherschwimmen können, bis ihr ursprüngliches spezifisches Gewicht wieder hergestellt ist.

Bei der durch die Versuche angeregten Gasausscheidung wurde nun regelmässig gleichzeitig eine beträchtliche Zunahme des Sauerstoffgehaltes der Blasengase beobachtet, s. Tabelle I (A. Moreau, 1877; Bohr, 1894), bei der Resorption der Blasengase eine besonders starke Abnahme des Sauerstoffgehaltes der Blasengase (Popta, 1910; Bohr, 1894; s. dagegen Greene, 1924). Durch mehrmalige Punktionen, immer gleich nach vollendetem Volumersatz kann der Sauerstoffgehalt sehr stark in die Höhe getrieben werden, allerdings wurde bis jetzt, auch unter Anwendung besonderer Versuchsbedingungen—Ausschaltung des Hauptresorptionsorgans (s. unten)—nie ein höherer Sauerstoffgehalt als 94 % z. B. bei *Serranus* erreicht (v. Ledebur, 1929). A. Moreau (1877) vermutete, dass reiner Sauerstoff sezerniert würde; der in der Blase gefundene Stickstoff sollte nachträglich in die Blase hineindiffundieren. Da dies, wie eben gezeigt wurde, häufig zur Erklärung des gefundenen Stickstoffgehaltes nicht ausreicht, und da es nicht gelingt, den Sauerstoffgehalt über einige 90 % zu erhöhen, wurde in der Folge dann von anderen Autoren (Bohr usw.) angenommen, dass der Sauerstoff bei diesen Änderungen des Blasen volumens zwar die Hauptrolle spielt, dass aber kein reiner Sauerstoff, sondern nur ein an Sauerstoff sehr reiches Gasgemisch in die Blase ausgeschieden wird, das auch noch die anderen im Wasser gelösten Gase enthält. Beobachtungen über Zunahmen des Stickstoffgehaltes nach Punktionen sind bis jetzt interessanterweise in keinem Falle bekannt geworden, obwohl nach dem oben über die Stickstoffsekretion ausgeführten ein derartiger Vorgang nicht von vornherein ausgeschlossen erscheint. Allerdings berechnete Popta (1910) aus Versuchen an *Tinca*, dass die durch Anhängen von Gewichten oder von Korkstückchen an die Fische hervorgerufenen Änderungen des Sauerstoffgehaltes der Blasengase nicht ausreichen, um die gemessenen Veränderungen des Blasen volumens zu erklären, es muss also auch Stickstoff bei den Versuchen in die Blase herein- und aus der Blase herausgekommen sein. In letzter Zeit ist dann hauptsächlich von Jacobs (1930) daraufhingewiesen worden, dass auch der Kohlensäuregehalt vorübergehend, besonders kurze Zeit nach der Punktion, stark ansteigen kann. Über diese Befunde wird weiter unten bei der Besprechung der darauf aufgebauten Theorien der Gassekretion näher eingegangen werden.

Die schon von den ersten Untersuchern (Biot, 1807; Delaroché, 1809; Conigliachi, 1811) gemachte Beobachtung, dass besonders die in grösserer Tiefe lebenden Meeresfische einen hohen Sauerstoffgehalt in der Schwimmblase besitzen (s. Tab. I), wurde von F. A. Moreau (1874) durch diese Versuchsergebnisse erklärt: die durch den Wasserdruck zusammengedrückten Blasengase werden durch die Ausscheidung von Sauerstoff auf das ursprüngliche Volumen gebracht. Dazu kommt nach Jacobs (1932), dass infolge des in grossen Tiefen besonders grossen Diffusionsverlustes von Gasen aus der Blase (wegen des höheren Blaseninnendruckes) auch bei ständigem Aufenthalt dort eine fortwährende Sekretion von Gas stattfinden muss. Es ist fraglich, ob diese Erklärungen ausreichend sind. Das Vorhandensein einer Reihe von Ausnahmen, d. h. von Tiefseefischen mit geringem Sauerstoffgehalt (z. B. Kilch) und von Oberflächenfischen mit hohem Sauerstoffgehalt hat Hüfner (1892) zu der Auffassung geführt, dass nur Fische, die rasch die Tiefe wechseln, ein aus Sauerstoff besonders reiches Gas ausscheiden, das wegen der grossen Aufnahmefähigkeit des Blutes gerade für Sauerstoff rasch herangebracht und rasch wieder fortgeschafft werden kann. Fische, die in gleichbleibender Tiefe leben, sollen mit der Sekretion von stickstoffreichen Gasen auskommen. Wie jedoch Winterstein (1921) betont, verlaufen die Sekretions- und Resorptionsvorgänge auch beim Sauerstoff für schnelle Änderungen des spezifischen Gewichtes bei plötzlichen Tiefenänderungen viel zu langsam. Dazu kommt, dass nach Scheuring (1922) bei Fischen, die längere Zeit in grösserer Tiefe gehalten wurden, ein Wiederabsinken des zuerst erhöhten Sauerstoffgehaltes beobachtet wurde. Es ist daher bis jetzt noch nicht möglich eine eindeutige Erklärung für den verschiedenen Sauerstoffgehalt in der Schwimmblase der verschiedenen Fische zu geben. Es ist dabei vielleicht auch der z. T. doch sehr wechselnde anatomische Aufbau der Gasausscheidungsapparate zu berücksichtigen.

Ein weiterer Beweis, dass es sich hier nicht um einfache Diffusionsvorgänge handeln kann, ist schliesslich die Tatsache der nervösen Beeinflussung der Gasausscheidung in die Schwimmblase. F. A. Moreau (1865) konnte als erster zeigen, dass die Gasausscheidung durch Nervenimpulse beeinflussbar ist. Durchschneidung der neben der Arteria mesenterica coeliaca verlaufenden sympathischen Nerven rief eine Erhöhung des Sauerstoffgehaltes der Schwimmblasengase bei der Schleie (von 6 % bis auf 27 % ca. drei Wochen nach der Operation) hervor. Da in der Arbeit nichts davon erwähnt ist, dass die Fische dabei zu leicht wurden, das Volumen der Blasengase also gleich blieb, muss bei Annahme einer vermehrten Gasausscheidung gleichzeitig eine vermehrte Gasabsorption (und zwar besonders von Stickstoff) stattgefunden haben. Die Möglichkeit, dass nur eine vermehrte Absorption von Stickstoff stattfand, konnte durch die Beobachtung ausgeschlossen werden, dass die Schwimmblase prall gefüllt bleibt. Nach Winterstein (1921) muss allerdings die Möglichkeit in Betracht gezogen werden, dass diese Wirkung der Nervendurchschneidung auch auf vasomotorischen Einflüssen beruhen kann (s. a. Hüfner). Diese Moreau'schen Versuche bedürfen allerdings wohl einer exakten Nachprüfung, besonders da die Gasanalysen vor und nach der Operation an verschiedenen Tieren ausgeführt wurden (s. a. Bohr, 1894).

Durch Durchschneidung der Rami intestinales des Vagus bei *Gadus callarius* kann nach Bohr (1894) eine Wiederauffüllung einer durch Punktion entleerten Schwimmlase für immer verhindert werden; anstatt der sonst zu beobachtenden Zunahme des Sauerstoffgehaltes der Blasengase war sogar eher ein Absinken des Sauerstoffgehaltes vorhanden (s. a. schon F. A. Moreau, 1876), eine Gasausscheidung findet dann nicht mehr statt. Diese Versuchsergebnisse wurden von Kuiper (1915) und Jacobs (1932) auch für andere Fischarten (Barsche) bestätigt. Nach chemischer Vagusreizung durch Pilocarpin-Injektionen beobachtete Dreser (1892) beim Hecht eine deutliche Zunahme des Sauerstoffgehaltes der Blasengase (im Durchschnitt von 23 % auf 39 %), allerdings handelt es sich dabei auch nur um Analysen bei verschiedenen Individuen. Es scheinen demnach im Vagus die zur Gasausscheidung notwendigen Nervenimpulse zur Schwimmlase zu gelangen, während im Sympathicus die die Gasausscheidung hemmenden Fasern verlaufen, bei deren Durchschneiden ein vermehrter Sauerstoffgehalt der Blasengase infolge Überwiegen der Vaguswirkung eintritt.

Die Einstellung des spezifischen Gewichtes der Fische erfolgt reflektorisch. Durch welche Reize dies hervorgerufen wird, ist bis jetzt noch nicht eindeutig sichergestellt. Nach Baglioni (1908) soll dabei hauptsächlich der Spannungszustand der Schwimmlasenwand, die von zahlreichen Nerven versorgt wird (Deineka, 1904), eine Rolle spielen. Jedenfalls sollen die kompensatorischen Schwimmbewegungen, die auftreten, wenn ein Fisch in eine andere Wassertiefe kommt, und dadurch eine Veränderung seines spezifischen Gewichtes durch Änderung seines Blasenvolumens entsteht, und die ihn in seine Ausgangslage zurückzubringen versuchen, dadurch hervorgerufen sein (s. a. Remotti, 1924). Ein kompensatorisches Abwärtsschwimmen wurde jedoch auch bei Volumverringern der Blase, also bei entdehnter Schwimmlasenwandung beobachtet, wenn die Fische dann künstlich durch Kork zu leicht gemacht wurden (Kuiper, 1915). Und auch Fische, denen die Schwimmlase operativ entfernt ist, versuchen dauernd an die Wasseroberfläche zu schwimmen (v. Frisch & Stetter, 1932). Nach v. Frisch (1934) ist zur normalen Regulation der Schwimmlasenfüllung eine normale Orientierung im Raume nötig: Fische ohne Augen und ohne Utriculus und Bogengänge besaßen meist eine zu wenig gefüllte Schwimmlase. Nach Meesters & Nagel (1934) soll die Gassekretion und die Gasresorption wahrscheinlich reflektorisch mit charakteristischen Flossenbewegungen zusammenhängen, die nach Jacobs (1935) durch die beim Absinken bzw. Steigen hervorgerufenen Flossenabbiegungen ausgelöst werden. Nur wenn diese Bewegungen ausgeführt werden können, soll die Sekretion bzw. Resorption zustande kommen.

IV. ANATOMIE UND PHYSIOLOGIE DER GASAUSSCHIEDUNGS- UND GASRESORPTIONSORGANE

Es wäre nun noch auf die Frage einzugehen, auf welches anatomische Substrat dieser Nerveneinfluss sich auswirkt, das heisst, ob besondere anatomische Gebilde in der Schwimmlase mit der Gasausscheidung in Zusammenhang gebracht werden

können. Das ist in der Tat der Fall, und zwar besonders deutlich in der Schwimmblase von Fischen, die über eine starke und schnelle Gasausscheidung (z. B. nach Punktionen) verfügen, speziell bei den Physoklisten. Es ist dies die von Joh. Müller (1840) entdeckte Schwimmblasendrüse. Dabei handelt es sich einmal um lokalisierte Anordnungen von Blutkapillaren, die schon den ersten anatomischen Beschreibern der Schwimmblase auffielen (Redi, 1684) und von Delaroche (1809) als "rote Körper" bezeichnet wurden, und ferner um aus dem Epithel der Blasenwandung entstandene Organe, die als "zellige Säume" und später als Gasdrüsen bezeichnet wurden. Zwischen den "roten Körpern" und den Gasdrüsen müssen enge funktionelle Beziehungen bestehen, da Gasdrüsen ohne "rote Körper" nicht vorkommen.

Bei den Fischen mit träger Gasausscheidung nach Volumverminderung des Blaseninhaltes, speziell bei Physostomen, die noch die Fähigkeit der Aufnahme atmosphärischer Luft in die Schwimmblase durch Luftschnappen besitzen, können diese lokalisierten Kapillaransammlungen fehlen, es ist nur ein diffuses Kapillarnetz in der Blase vorhanden; auch die eng lokalisierten drüsigen Organe fehlen. Dafür ist die ganze Schwimmblase von einem mehr oder weniger hohen Epithel ausgekleidet, dem die Fähigkeit der Gassekretion zugeschrieben werden muss.

In diesem Zusammenhang ist noch kurz etwas über die Gasfüllung der Schwimmblase bei den Physostomen zu berichten. Es ist zwar immer wieder bestritten worden, dass beim Luftschnappen Gase durch den in der Regel sehr engen Ductus pneumaticus in die Blase gelangen können, aber durch neuere Untersuchungen (s. a. die Versuche an Jungfischen: v. Ledebur, 1928) ist diese Tatsache wohl sicher gestellt. Evans & Damant (1928) konnten beim Goldfisch, Jacobs (1934) bei Salmoniden (*Hucho*, *Salmo*), Cypriniden (*Phoxinus*, *Tinca*, *Cyprinus*) und bei *Esox lucius* eindeutig nachweisen, dass eine Neufüllung der Schwimmblase durch Luftschlucken möglich ist. Verhindert man jedoch, dass die Fische nach der Blasenentleerung an die Wasseroberfläche zum Luftschnappen kommen, dann findet trotzdem, jedenfalls bei einer Reihe von Physostomen ein Volumersatz der entfernten Blasengase statt, der dann ebenso wie bei den Physoklisten (es liegen ja jetzt dieselben Bedingungen vor) nur durch eine Gassekretion zustande kommen kann. Schon von A. Moreau (1877) und Hübner (1892) wurde bei *Tinca* und *Esox* eine derartige Gassekretion festgestellt, von Jacobs (1934) dann auch noch bei der Elritze und beim Karpfen. Bei den Salmoniden fehlte die Fähigkeit der Gassekretion. Die Gasausscheidung ist in der Regel beträchtlich langsamer als bei den Physoklisten, beim Hecht, bei den Cypriniden, besonders aber beim Karpfen dauert es oft 7–10 Tage und länger, bis das ursprüngliche spezifische Gewicht wieder erreicht ist. Auch hier nimmt, wie bei den Physoklisten, hauptsächlich der Sauerstoffgehalt nach einer Blasenentleerung stark zu, bei *Phoxinus* im Durchschnitt auf 46.9 %, bei *Esox* von 32 % auf 46 %, bei der Schleie von 1.9 % auf 22.9 %, beim Karpfen allerdings nur von 3.8 % auf 6.2 %. Nach Jacobs ist dabei, ebenso wie beim Barsch auch der Kohlensäuregehalt besonders während des Volumersatzes stark erhöht, der z. B. beim Hecht von im Durchschnitt 6.1 % auf 15.1 % anstieg. Aus den Versuchen ging ferner hervor, dass auch dann, wenn die Tiere (Hechte und Cypriniden)

Gelegenheit zum Luftschnappen haben, meist gleichzeitig die Gassekretion in Gang gebracht wird.

Bei stärkerer Erniedrigung des Aussendruckes (Evacuieren)—bei Rotfedern bei einer Druckerniedrigung von 30 mm. Hg. (v. Kokas, 1932)—kann bei den Physostomen eine Entleerung der Schwimmlase durch Austreten von Gasen durch den Ductus pneumaticus zustande kommen, der gewissermassen als Sicherheitsventil, als Ersatz für das bei den Physoklisten als Resorptionsorgan dienende “hintere Gefässorgan” (s. unten) vorhanden ist (Jäger, 1903, 1904). Allerdings kann es auch trotz Vorhandenseins eines Luftganges wahrscheinlich wegen Versagens dieser Vorrichtung bei zu starken und zu plötzlichen Druckerniedrigungen zu den Erscheinungen der “Trommelsucht” kommen (z. B. Kilch nach Hüfner). Ob auch bei den normalerweise sehr geringfügigen Druckunterschieden während des Lebens der Fische Luft auf diese Weise austreten kann, ist nach Jacobs immerhin fraglich und müsste erst noch untersucht werden.

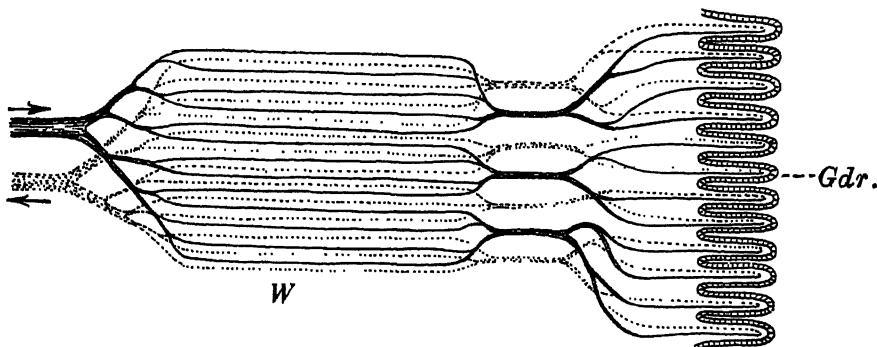


Abb. 1. Schema des Blutkreislaufes im arterio-venösen Wundernetz der Gasdrüse des Aales (nach Woodland, 1911). *W*, Wundernetz; *Gdr.*, Gasdrüse. Arteriolen und arterielle Kapillaren = ausgezogene Linien. Venolen und venöse Kapillaren = gepunktete Linien.

Was nun zunächst die “roten Körper” betrifft, so sind dieselben besonders eingehend von Woodland (1911) beschrieben worden. Es handelt sich dabei um komplizierte, sehr regelmässig aufgebaute Wundernetzbildungen, deren äussere Gestalt sehr wechseln kann, deren wesentlicher Aufbau jedoch immer der gleiche ist (s. Abb. 1). Die die Blase versorgende Arterie teilt sich in der Regel in mehrere Äste, von denen jeder sich nach kurzem Verlaufe plötzlich in eine sehr grosse Anzahl gerade gestreckter, untereinander parallel verlaufender Kapillaren aufsplittet. Diese Kapillaren sammeln sich entweder wieder zu grösseren Gefässen, die nach kurzem Verlaufe sich in Endkapillaren verzweigen und die Zellen der Gasdrüse versorgen. Oder aber es fehlt diese Wiedervereinigung, die Wundernetzkapillaren gehen direkt in die Gasdrüsenkapillaren über. Die aus der Gasdrüse austretenden venösen Kapillaren gehen ebenfalls direkt oder nach kurzer Vereinigung zu grösseren Gefässen unter Wiederaufteilung in gerade Kapillaren in das distale Ende der “roten Körper” über, in denen dann also dicht nebeneinander und parallel arterielle und venöse Kapillaren verlaufen. Am proximalen Ende des Wundernetzes vereinigen sich dann die venösen

Kapillaren zu einer oder mehreren abführenden Venen. In der Regel ist eine venöse Kapillare, die nach Woodland ein besonders verdicktes Endothel besitzt, von mehreren etwas engeren arteriellen Kapillaren umgeben. Ähnlich wie in einem Rückflusskühler fliesst also hier das zur Gasdrüse fließende und das von dort kommende Blut dicht nebeneinander auf eine längere Strecke in entgegengesetztem Sinne aneinander vorbei. Krogh (1929) hat beim Aal die Grössenverhältnisse dieser Kapillaren besonders eingehend untersucht; er fand, dass diese Kapillaren 4 mm., d. h. etwa 8 mal so lang sind wie die längsten Kapillaren des übrigen Körpers, die Muskelkapillaren. Das Gesamtvolumen der beiden Kapillarsysteme betrug 64 cmm., d. h. also ungefähr die Grösse eines Wassertropfens, in denen 88000 venöse und 116000 arterielle Kapillaren vorhanden sind, mit einer Gesamtlänge von 352 bzw. 464 m. Die venösen und arteriellen Kapillaroberflächen wurden auf 106, bzw. 105 cm.² berechnet. Es ist also hier auf kleinstem Raume eine riesige Oberflächenvergrößerung der Blutbahn vorhanden, wodurch Stoffaustauschvorgänge zwischen dem arteriellen und venösen Blut weitgehend begünstigt werden (s. unten).

Während die Wundernetzbildungen immer durch mehr oder weniger Gewebe von dem Blasenlumen getrennt sind und bei manchen Fischen sich sogar etwas entfernt von der Schwimmblase befinden, bilden die Gasdrüsen, von wenigen Ausnahmen abgesehen (z. B. Esociden, Cypriniden), die innerste Schicht der Blasenwandung, aus deren Epithel sie entstanden sind (Reis, 1906). Die Anordnung der Drüsenzellen kann bei den verschiedenen Fischarten sehr verschiedenartig sein (s. Abb. 2). Das Drüsenepithel kann (Reis, 1906; Woodland, 1911) entweder das ganze Innere des Blasenlumens auskleiden oder nur einen kleinen Teil der Blaseninnenfläche einnehmen. Die Zellen können nur eine einfache glatte Lage bilden, oder aber das Drüsenepithel bildet an eng umschriebenen Stellen einfache oder zusammengesetzte in das Blasenlumen vorspringende Falten, und schliesslich können die Zellen vielschichtig, in mehr oder weniger dicken, kompakten Massen angeordnet sein, die nach dem Blasenlumen zu von einem einschichtigen Plattenepithel überdeckt sein können, und in welchem die tiefer gelegenen Zellen mit dem Blasenlumen durch ein System von anastomosierenden, an der Oberfläche der Drüse sich in die Blase öffnenden Kanälchen (interzelluläre Lumina) kommunizieren. Jacobs (1930) konnte jedoch bei *Perca* niemals Ausführungsgänge oder Kanäle beobachten. Nach Reis (1906) stellt dieser Übergang von glatten Epithel bis zu kompakten Drüsen eine fortschreitende Vervollkommenung der Gasdrüse dar, der in gewissem Grade auch eine Zunahme in der Geschwindigkeit der Gassekretion entspricht (F. A. Moreau, 1876).

Die feinere Struktur der Gasdrüsenzellen selbst ist häufig untersucht worden (Nusbaum, Reis, Woodland) und zuletzt von Jacobs (1930) beim Flussbarsch (*Perca*) eingehend beschrieben worden. Nach Jacobs können in der Gasdrüse, besonders bei Fixierung mit Osmiumsäurehaltigen Fixierungsmitteln mehrere Zellformen unterschieden werden, die alle durch Übergänge miteinander verbunden sind, und deren Struktur zu der Annahme Anlass gab, dass es sich um typische Drüsenzellen handelt. Wie in anderen Drüsenzellen treten zuerst in Kernnähe Granula auf, die an Grösse zunehmen und sich schliesslich kurz vor der Ausstossung

aus der Zelle zu einer leichter flüssigen Substanz auflösen. Es entstehen sogenannte Blaszellen mit einer oder mehreren "Sekretvacuolen", die im fixierten Präparat meist mehr oder weniger leer erscheinen, vor ihrer völligen Auflösung jedoch

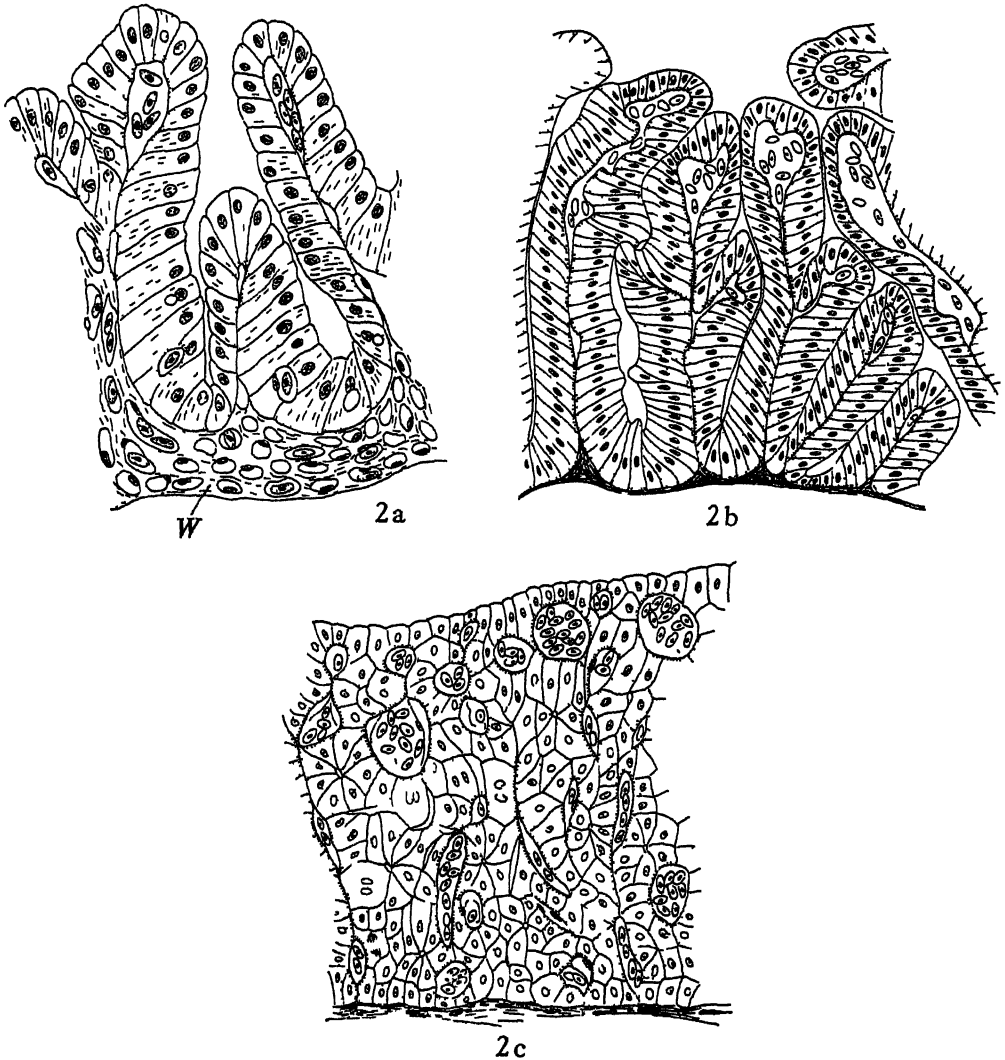


Abb. 2. Verschiedene Formen von Gasdrüsen (nach Woodland, 1911). a. *Gobius niger* ($\times 330$). Einfache Faltungen des Schwimmlasenepithels, dicht unter dem Epithel die quergeschnittenen Kapillaren des Wundernetzes (W). b. *Trigla hundo* ($\times 250$). Komplizierte Faltungen des Epithels. c. *Smaris maurii* ($\times 250$). Kompakte, vielschichtige Gasdrüse.

verschiedenartigste Inhaltskörper, besonders oft Fettkugeln zeigen. Nach Jacobs wird das gebildete flüssige Sekret nicht in die Schwimmlase abgeschieden, denn das Innere der Blase wurde von ihm entgegen den Befunden von Vincent & Barnes (1896), die ein mitunter recht reichliches, die Drüsen überziehendes Sekret feststellen konnten, frei von Flüssigkeit gefunden. Sondern das Sekret soll

aller Wahrscheinlichkeit nach an die sehr zahlreichen Blutkapillaren im Drüsengewebe unter Filtration durch die Zellwand abgegeben werden. Die Gasdrüse wird also als eine Drüse mit innerer Sekretion aufgefasst. Gasbläschen wurden von Jacobs niemals, auch nicht nach Anregung der Gasausscheidung durch Blasenpunktion, in den Drüsenzellen beobachtet. Er nimmt an, dass es sich bei dem von anderen Autoren (Reis & Nusbaum, 1905–6; Woodland, 1911; Powers, 1932) beschriebenen Vorkommen von "Gasvakuolen" in den Gasdrüsenzellen—auf Grund dieser Tatsache wurden besondere Theorien über die Gasausscheidung aufgestellt (s. unten)—um jene oben erwähnten Flüssigkeitsvakuolen handelt, die besonders bei Fixierung mit Bouin, Carnoy oder Zenker optisch leere kugelige Gebilde darstellen. Schon Vincent & Barnes (1896) und Jäger (1903) hielten diese kugeligen Gebilde für Flüssigkeit enthaltende Vakuolen und auch Woodland (1912) konnte in einer späteren Arbeit durch mikroskopische Untersuchungen der überlebenden Drüse zeigen, dass es sich um Flüssigkeitsvakuolen handelt und dass sicher keine Gasbläschen, auch nicht in den tätigen Gasdrüsenzellen, vorkommen.

Bevor wir nun näher auf die mit diesen Befunden schon gestreiften Theorien über die Gasausscheidung eingehen, sind noch kurz andere in der Hauptsache auch aus Blutkapillaren bestehende Gebilde zu beschreiben, die nur in der Schwimmblase von Physoklisten vorkommen. Es ist das jenes von Jäger (1903) zuerst beschriebene und als Gasresorptionsorgan erkannte "Oval". Bei diesen, von Rauther (1923) auch als "hintere Gefässorgane" bezeichneten Bildungen handelt es sich um das Vorhandensein eines sehr dichten Kapillarnetzes, das durch die plötzliche büschelförmige Verzweigung von Intercoalararterien (seltener auch der Arteria coeliaca) entsteht und meist in den dorsalen und hinteren Teilen der Schwimmblase sich befindet, im Gegensatz zu den also räumlich schon scharf getrennten, in den vorderen und ventralen Blasenteilen gelegenen aus der Arteria coeliaca stammenden Gasdrüsenkapillaren und- Wundernetzen (s. Abb. 3). Diese Kapillaren liegen dabei dicht unter dem die Blase auskleidenden Epithel. Diese dünnwandigen und gefässreichen Blasenteile sind von dem vorderen, die Gasdrüsen enthaltenden, dickwandigen Blasenteil durch ein Muskeldiaphragma getrennt, das entweder in das Blasenlumen vorspringt und die Blase in eine vordere und hintere Kammer teilt (z. B. *Serranus*), oder aber dicht dem "hinteren Gefäss-

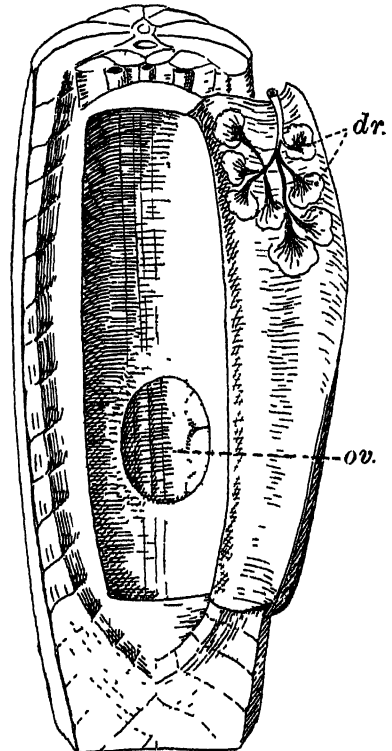


Abb. 3. Schwimmblase von *Perca fluviatilis* (nach Jacobs, 1935). Ventral aufpräpariert. *dr*, Gasdrüsen (Wundernetze und "zellige Saume"), vorn-ventral gelegen; *ov*, Oval, halbgeöffnet; dorsalhinten gelegen.

und hintere Kammer teilt (z. B. *Serranus*), oder aber dicht dem "hinteren Gefäss-

organ" aufliegt und das sogenannte "Oval" bildet (z. B. *Perca*, *Lota*, *Gadus*) (Woodland, 1913). Durch verschieden weite Öffnung des im Diaphragma enthaltenen, die zentrale Öffnung desselben umschliessenden Sphincters kann eine mehr oder weniger starke Kommunikation der Gase der vorderen Kammer mit denen der hinteren Kammer hergestellt werden, in der durch Diffusion ein Gasaustausch mit den dicht unter dem Epithel liegenden Gefässen stattfindet, der infolge des höheren Gasdruckes der Blasengase zu einer Resorption derselben führen muss. Liegt die aus Muskeln, elastischen Fasern usw. bestehende und wenig Gasdurchlässige Diaphragmamembran dem "hinteren Gefässorgan" dicht auf (Oval), so kann durch mehr oder weniger weite Öffnung des Sphincters ein verschieden grosses Gebiet von Kapillaren, die nur von Gasdurchlässigem Epithel bedeckt sind, mit den Blasengasen in Gasaustausch treten und Gase resorbiert werden. Ausser den circulären Muskelfasern des Sphincters wurden beim Oval von Nusbaum & Reis (1905-6) auch noch radiär angeordnete, wohl zur Erweiterung des Ovals dienende Muskelfasern beobachtet.

Nusbaum & Reis konnten zuerst direkt eine Erweiterung des Ovals nach dem Tode der Fische und Eröffnung der Schwimmlase feststellen, die besonders rasch sofort nach dem Tode eintrat, bei Eröffnung der Blase eine Stunde nach dem Tode war die Erweiterungsbewegung stark verlangsamt. Woodland (1912) beobachtete, dass das sonst geöffnete Oval von *Gadus* 1-2 Stunden nach Anregung der Gasausscheidung durch Anhängen eines Gewichtes an einen Fisch meist völlig geschlossen war. Nach Meesters & Nagel (1934) öffnet sich das Oval sehr schnell (in wenigen Sekunden) nach dem Tode des Fisches. Erst, als es gelang, durch besondere Bedingungen (Urethannarkose, niedrige Temperatur und Eröffnung der Blase innerhalb 30 Sekunden) diese sonst auftretenden postmortalen Öffnungsbewegungen stark zu verlangsamen, konnte regelmässig gezeigt werden, dass bei Gasresorbierenden Barschen (durch Anhängen von Kork zu leicht gemachte Fische) das Oval in der Tat weit geöffnet ist, bei durch Anhängen von Blei zu schwer gemachten, sezernierenden Fischen dagegen ganz oder fast ganz geschlossen ist. Dass es sich in der Tat bei diesen "hinteren Gefässorganen" um das Hauptresorptionsorgan der Schwimmlase handelt, konnte durch die operative Ausschaltung dieses Organes bei *Serranus* (Zubinden der zentralen Diaphragmaöffnung), wobei der vordere Schwimmlasenteil mit der Gasdrüse intakt bleibt, gezeigt werden (v. Ledeber, 1929). Die Mehrzahl der so operierten Fische wurde nach einiger Zeit zu leicht und wurde immer wieder passiv, trotz nach unten gerichteter Schwimmbewegungen an die Wasseroberfläche getrieben.

Aus dieser Tatsache und der regelmässigen Wiederfüllung der Blase nach Punktionen konnte geschlossen werden, dass die Gasausscheidung in der Tat in der Gasdrüse stattfindet und dass diese Sekretion kontinuierlich und unbeeinflusst von nervösen Regulationen (Dehnung der Blasenwand, Flossenbewegungen usw.) stattfindet. Diese kontinuierliche Gasausscheidung ist notwendig, um den dauernden Gasverlust durch Diffusion aus der Schwimmlase zu decken, der in der Hauptsache, wie diese Versuche zeigen, durch das "hintere Gefässorgan" stattfindet. Das Oval wurde von Woodland (1912) normalerweise

auch dauernd geöffnet gefunden. Es muss also ein dauernder Gasstrom durch die Blase vorhanden sein. Wird der Abstrom wie in diesen Versuchen gedrosselt, muss es zur Volumzunahme der Blase kommen. Auch die Ergebnisse anderer Versuche bestätigen dies: Ausschaltung der Gasdrüse durch Abbindung des zur Schwimmblase führenden Gefässstieles, der die zu den Wundernetzen ziehenden Gefässe und Nerven der Schwimmblase enthält (v. Ledebur, 1929), oder durch Durchschneidung der Rami intestinales des Vagus (Jacobs, 1932) verhindert nicht nur den Volumersatz nach Punktion, sondern ruft infolge der fortdauernden kontinuierlichen Resorption bei jetzt fehlender Gassekretion ein Zuschwerwerden der Fische, eine Volumabnahme der Blasengase hervor, die um so grösser ist, je stärker der Druck der Blasengase ist. Dabei soll jedoch merkwürdigerweise nach Bohr (1894), der deshalb das Vorhandensein einer Gasdiffusion durch die Blasenwandung und einer kontinuierlichen Gassekretion ablehnt, ein durch Punktion hervorgerufener hoher Sauerstoffgehalt der Blase lange Zeit erhalten bleiben. Zu dieser kontinuierlichen Gasausscheidung würde dann im Bedarfsfalle (bei Erhöhung des Aussendruckes, nach Punktionen usw.) eine zusätzliche, nervös regulierte Tätigkeit der Gasdrüse hinzukommen.

Schon die Tatsache der Abhängigkeit der Resorptionsgrösse von dem Gasdruck lässt darauf schliessen, dass es sich hier um eine rein physikalisch erklärbare Diffusion handelt. Dafür sprechen neben dem Fehlen besonderer histologischer Strukturen auch andere Versuchsergebnisse. Die Resorptionsgeschwindigkeit der einzelnen Gase entspricht ihrer Diffusionsgeschwindigkeit: Kohlensäure wird ausserordentlich rasch resorbiert (Jacobs, 1932; Meesters & Nagel, 1934), Sauerstoff bedeutend langsamer, Stickstoff fast garnicht. Volumvergrösserung der Blase durch Injektion von Sauerstoff wird rascher durch Resorption ausgeglichen als die Vergrösserung durch Luftinjektion, wobei bei letzterer der Stickstoffgehalt der Blasengase infolge der überwiegenden Sauerstoffresorption zunimmt (Baglioni, 1908; Popta, 1910). Diese Resorptionsvorgänge, auch die des Sauerstoffs, verlaufen sehr langsam: trommelsüchtige Fische aus grösserer Meerestiefe brauchen Tage trotz des hohen Druckes der Blasengase, bis ihr durch die plötzliche Druckentlastung stark vergrössertes Schwimmblasenvolumen wieder normal geworden ist.

Als Hauptdurchtrittsort der Gase durch die Schwimmblasenwand bei diesen Diffusionsprozessen kommt schon wegen der Dünnhheit der Wandung und der ausgedehnten Kapillargebiete hauptsächlich das Oval oder "hintere Gefässorgan" in Betracht. Daneben werden Diffusionsvorgänge, allerdings in viel geringerem Masse auch durch die übrige Blasenwandung hindurch stattfinden. In getrocknetem Zustande soll die Schwimmblase fast völlig gasdicht sein, derartig trockene, gefüllte Blasen enthielten noch nach 4 Monate langer Aufbewahrungszeit einen Sauerstoffgehalt, der weit über dem der atmosphärischen Luft lag (über 53 %) (Popta, 1910; s. a. Biot, 1807). Auch nach Bohr (1892) soll die herauspräparierte und mit Luft gefüllte Schwimmblase von *Esox*, die in reinen Sauerstoff gehängt wurde, keinen Sauerstoff von aussen nach innen durchlassen. In eigenen (unveröffentlichten) Versuchen, in denen die Gasdiffusion durch die isolierte innere, sich leicht von den anderen Teilen der Blasenwandung ablösende Schwimmblasen-

schicht, aus dem Blasenepithel und der darunter gelegenen Gefässschicht bestehend, im feuchten Zustande untersucht wurden, ergab sich eine Durchlässigkeit für alle Gase in beiden Richtungen. Während aber beim Sauerstoff und besonders beim Stickstoff erst nach Stunden ein merklicher Gasaustausch zu beobachten war, drang die Kohlensäure in wenigen Minuten von aussen durch die Blasenwandung hindurch, etwas langsamer in umgekehrter Richtung. Mit Stickstoff oder Sauerstoff etwas gefüllte Säcke aus Blasenwandung blähten sich wenige Minuten, nachdem sie in Kohlensäure gebracht wurden, auf und konnten auf diese Weise leicht zum Platzen gebracht werden. Umgekehrt mit Kohlensäure gefüllte Blasen sinken allmählich, in Stickstoff- oder Sauerstoffreiche Atmosphären gebracht, zusammen. Die sehr viel grössere Diffusionsgeschwindigkeit der Kohlensäure, besonders in der Richtung von aussen nach innen, gegenüber der des Sauerstoffs und des Stickstoffs wird auf diese Weise besonders klar (s. a. Wertheimer, 1925).

Dass diese Diffusion durch die Blasenwandung auch beim intakten Tier in beiden Richtungen verlaufen kann, zeigen Versuche an Fischen, deren Gasdrüse durch Vagusdurchschneidung ausgeschaltet war, und bei denen entsprechend dem Diffusionsgefälle Sauerstoff oder Kohlensäure nach aussen, Stickstoff jedoch in die Schwimmlase einströmte, weil durch künstliche Gasmischungen mit Sauerstoff und Kohlensäure in der Blase die Stickstoffspannung in der Schwimmlase gegenüber der der Umgebung sehr klein gemacht worden war. Durch Erhöhung des Kohlensäuregehaltes des Wassers (Hall, 1924) ebenso wie durch Erhöhung des Wasserstoffgehaltes (Traube-Mengarini, 1888–1889) durch kontinuierliches Durchleiten der betreffenden Gase durch die Aquarien konnte ein Übertritt dieser Gase in die Blase (bis zu 85 % Wasserstoff) hervorgerufen werden. Auch hierbei handelt es sich wohl um einfache Diffusionsvorgänge, wobei allerdings nichts über die Durchtrittsstelle ausgesagt ist; auch im Gebiet der Kapillaren der Gasdrüse werden diese Gase in die Blase hineindiffundieren können.

V. THEORIEN ÜBER DIE GASSEKRETION

Es wäre nun noch etwas über die Theorien zu sagen, die von den verschiedenen Forschern zur Erklärung der, wie wir oben gesehen haben, sicher vorhandenen Ausscheidung von Gasen in die Schwimmlase entgegen dem Diffusionsgefälle aufgestellt worden sind. Zwei verschiedene, sich gegenüberstehende Ansichten sind dabei geäussert worden. Während auf der einen Seite angenommen wird, dass tatsächlich in den Gasdrüsenzellen entsprechend ihrer Lage als ein die Blase auskleidendes Epithel die "Gasverdichtungsarbeit" stattfindet, wird auf der anderen Seite, besonders neuerdings, der Gasdrüse nur die Fähigkeit der Bildung eines besonderen Sekretes zugeschrieben, das in die Blutkapillaren abgeschieden wird, und in diesen, hauptsächlich in den Wundernetzen, eine Erhöhung der Gasspannung hervorrufen soll, die schliesslich zu einer Gasdiffusion in die Blase führt.

Nur ganz kurz wäre hier noch eine neuerdings von Leiri (1935) aufgestellte Theorie der Sauerstoffsekretion zu erwähnen, die das Vorhandensein der Gasdrüse überhaupt ganz vernachlässigt. Nach Leiri wird der an das Eisen des Hämoglobin

magnetisch gebundene Sauerstoff in Freiheit gesetzt durch starke magnetische Felder, die infolge des durch den Kreislauf hervorgerufenen Strömungspotentials (und der daraus entstehenden elektrischen Ströme) besonders in den Kapillaren vorhanden sein sollen. Der Zweck der Wundernetze wäre eine Erhöhung des Strömungspotentials und damit der den Sauerstoff freimachenden magnetischen Kräfte.

Die erste Anschauung wurde besonders von Nusbaum und Reis (1905-7) angenommen und durch die Beobachtung dieser Autoren gestützt, die die in den Gasdrüsenzellen gefundenen Vakuolen für Gasbläschen hielten. Diese Gasblasen sollten unter chemischer Umwandlung eines mehr oder weniger grossen Teiles des Protoplasmas und besonders auch des Zellkernes der Gasdrüsenzelle entstehen, und entweder direkt aus den Zellen in die Schwimmblase abgegeben werden—die Zelloberfläche war nicht selten durch kugelige Hohlräume gegen das Blasenlumen vorgewölbt—oder aber durch die in den kompakten Drüsen vorkommenden Ausführungsgänge. Ausserdem soll noch ein körniges, flockiges Sekret, das als Zwischenprodukt aufgefasst wird, ausgeschieden werden (s. a. Woodland, 1912), aus Zellzerfallsprodukten bestehend (Vincent & Barnes (1896) konnten in ihm ein Nukleoproteid nachweisen), aus dem dann später in der Blase die Gase freiwerden. Das Gas selbst soll aus Sauerstoff und Stickstoff bestehen. Über die Art der chemischen Umwandlungsprozesse in den Zellen wurde nichts ausgesagt, und auch auf den Zweck und den Sinn der Wundernetzbildung, die doch schon wegen ihres konstanten Zusammentreffens mit den differenzierten Gasdrüsen eine wichtige Bedeutung besitzen müssen, wird von diesen Autoren nicht eingegangen. Durch die genauen Nachuntersuchungen von Woodland (1912), der wie oben erwähnt, feststellen konnte, dass es sich bei den kugeligen Hohlräumen in den Gasdrüsenzellen nicht um Gasblasen, sondern um Flüssigkeitsvakuolen handelt, wurde dieser Theorie, jedenfalls in der von Nusbaum & Reis angenommenen Form eine wichtige anatomische Grundlage genommen. Es bleibt aber meiner Ansicht nach sehr wohl die Möglichkeit bestehen, dass das in den Gasdrüsen gebildete und wohl sicher mehr oder weniger flüssige Sekret in die Schwimmblase ausgeschieden wird, und dort in gasförmiger Produkte zerfällt.

Dagegen beruhen die anderen Theorien auf der Vorstellung, dass dies in den Gasdrüsenzellen gebildete Sekret an das Blut abgegeben wird und dort, besonders in den Wundernetzen auf irgend eine Weise den Sauerstoff aus dem Oxyhämoglobin freimacht, der dann auf dem Diffusionswege in die Blase gelangen soll. Nach diesen Theorien ist also die Gasdrüse selbst garnicht mehr das Organ, in der die Erhöhung der Gasspannungen erfolgt, bis schliesslich eine Gasausscheidung in die Blase hinein stattfinden kann, sondern dieser Vorgang läuft in den Kapillaren der Wundernetze ab. Ganz allgemein ist dabei zu bedenken, dass es einmal Schwimmblasen gibt, die keine Wundernetze besitzen und trotzdem, allerdings sehr viel träger Gase ausscheiden können. Und dann darf meines Erachtens nicht die ganz regelmässige und typische Lage der Gasdrüse in allen Schwimmblasen unberücksichtigt gelassen werden. Die Gasdrüsenzellen stellen immer den innersten Teil der Schwimmblasenwandung dar, sie kleiden als einschichtiges Epithel glatt oder in komplizierten, in

das Blasenlumen sich vorwölbenden Falten oder aber schliesslich in mehrschichtigen Zelllagen die Blase in mehr oder weniger grossem Masse innen aus. Es ist nicht ohne weiteres verständlich, warum ein seiner Anordnung und anatomischen Lage nach besonders für die Ausscheidung von Substanzen in die Blase geeignetes Organ nichts direkt mit der Gasausscheidung zu tun haben soll, sondern nur ein Sekret von dem Blasenlumen weg in die Kapillaren abgeben soll, während das nach diesen Vorstellungen für die Gasausscheidung selbst in Betracht kommende Organ, die Wundernetze, vom Lumen der Blase zum mindesten durch die Gasdrüsenzellen selbst getrennt ist.

So nahm Jäger (1903–6) und zuerst auch Reis und Nusbaum (1905) und Woodland (1911) die Bildung eines die Erythrocyten zerstörenden Toxins in der Gasdrüse an, das in den Kapillaren durch Auflösung der roten Blutkörperchen den an das Hämoglobin gebundenen Sauerstoff freimachen soll. Zerfallsprodukte von Erythrocyten konnten von ihm in grosser Menge in den Kapillaren eines lange Zeit konserviert aufgehobenen Präparates nachgewiesen werden. In späteren Untersuchungen konnte dies jedoch nicht bestätigt werden (Jacobs, 1930).

Dass die Jaeger'sche Annahme nicht haltbar ist, zeigten schon Berechnungen von Bohr (1905), s. a. Winterstein (1921): bei einer beobachteten Ausscheidung von 10 cc. Sauerstoff während 6 Stunden bei einem 1 kg. schweren Dorsch müssten alle Erythrocyten und die gesamte Hämoglobinmenge des Tieres zerstört und wieder neu gebildet worden sein, ein Vorgang, der wohl kaum angenommen werden kann. Woodland (1912) konnte ferner in dem Extrakt aus tätigen Gasdrüsen keine Substanz finden, die Erythrocyten zerstört und Sauerstoff aus dem Oxyhämoglobin freimacht.

Haldane (1922) hat dann zuerst auf eine im physiologischen Bereich liegende Möglichkeit hingewiesen, wie eine in den Drüsenzellen gebildete Substanz eine Erhöhung der Sauerstoffspannung des Blutes hervorrufen kann, nämlich die Kohlensäure. Nach Barcroft & King (1909) ist bei niedrigen Temperaturen der Einfluss der Kohlensäure in Bezug auf die Fähigkeit, Sauerstoff aus dem Oxyhämoglobin auszutreiben, relativ grösser als beim warmblütigen Organismus; die Dissoziation des Oxyhämoglobins ist beim Kaltblüter daher weniger vom Abfall des Sauerstoffdruckes als von der Erhöhung des Kohlensäuredruckes abhängig. Und von Krogh & Leitch (1919), Root (1931), Willmer (1934) konnte gezeigt werden, dass das Sauerstoffbindungsvermögen des Blutes, besonders von Frischwasserfischen durch Kohlensäure stark herabgesetzt wird. Die Kohlensäure des venösen Blutes soll, nach Haldane, in den Wundernetzen, deren Bedeutung ja wohl in der sehr starken Verlangsamung des Blutstromes und der damit verbundenen Möglichkeit eines grossen Diffusionsaustausches zwischen den dicht benachbarten arteriellen und venösen Kapillaren besteht, in das arterielle Blut herüber diffundieren, vielleicht auch aktiv in die arteriellen Kapillaren sezerniert werden, wofür die besonders verdickten Endothelien in den venösen Kapillaren sprechen würden (s. oben). Durch das Ansteigen der Kohlensäurespannung in dem die Gasdrüsenzellen versorgenden Blut würde auch die Sauerstoffspannung desselben ansteigen und damit den Drüsenzellen ihre, von Haldane angenommene Sauerstoffsekretionsarbeit erleichtern.

Dieser von Haldane angedeutete Weg einer Freimachung von Sauerstoff dadurch, dass durch Kohlensäure, oder allgemeiner ausgedrückt, durch Erhöhung der H-Ionenkonzentration eine Zunahme der Dissociation des Oxyhämoglobins eintritt, ist die Grundlage für eine neuerdings von verschiedenen Autoren aufgestellte Theorie der Gasausscheidung geworden. Hall (1924) konnte zeigen, dass das Dialysat einer durch Punktion oder Druckerhöhung zur Sekretion angeregten Gasdrüse saurer reagiert als das einer ruhenden Drüse: der kolorimetrisch gemessene pH-Wert war 2 Stunden nach der Punktion von 7·05 auf 6·38, 4 Stunden nach einer Druckerhöhung von 6·96 auf 6·64 abgesunken. Nach Hall soll diese in der Gasdrüse gebildete, ihrer Natur nach unbekannte Säure in die Kapillaren diffundieren und dort eine Zunahme der Sauerstoffspannung durch Vermehrung der Dissoziation des Oxyhämoglobins hervorrufen.

Neuerdings hat dann Jacobs (1930-4) (s. a. Koch, 1934) diese Gedankengänge aufgenommen und eine Arbeitshypothese über die bei der Gasausscheidung in die Schwimmblase stattfindenden Vorgänge aufgestellt. Nach Jacobs findet gar kein Gassekretionsprozess im engeren Sinne des Wortes statt, sondern die Gasdrüse sezerniert eine in die Kapillaren diffundierende Flüssigkeit, die, ausser einer wahrscheinlich kapillarerweiternden Wirkung—die Blutgefäße der tätigen Gasdrüse sind in der Tat stark erweitert, die Zahl der offenen Kapillaren nimmt während der Sekretion stark zu—die Fähigkeit besitzt, die Spannung der Kohlensäure des Blutes zu erhöhen, die dann in die Blase hineindiffundieren kann. Diese Zunahme der Kohlensäurespannung bewirkt nun aber gleichzeitig eine Zunahme der Sauerstoffspannung im Blut infolge verstärkter Dissoziation des Oxyhämoglobins. In den Wundernetzen wird in der Hauptsache ein Ausgleich dieser verschiedenen Gasspannungen des der Drüse zufließenden und aus der Drüse wegfließenden Blutes stattfinden, in dem Sinne, dass Kohlensäure und Sauerstoff aus den venösen in die arteriellen Kapillaren herüberdiffundieren. Durch erneute Sekretion in das Blut und wiederholte Erhöhung der Gasspannungen und Diffusion der Gase “kommt durch die Einschaltung des Wundernetzes in den Blutzirkulationsapparat ein Kreislauf der Gase in dem betreffenden Organsystem zustande, der nach Bedarf solange läuft, bis in dem Gasdrüsenblut die Gasspannung erreicht ist, die für eine Gasdiffusion in die Schwimmblase hinein jeweils erforderlich ist” (Jacobs, 1930, S. 624).

Entsprechend dieser Theorie konnte Jacobs in Versuchen am Flussbarsch feststellen, dass die Kohlensäure bei der Zusammensetzung der nach einer Punktion in die Blase ausgeschiedenen Ersatzgase besonders dann eine wichtige Rolle spielt, wenn der Gasersatz schnell erfolgt. Analysen der Blasengase nach fast oder gerade vollendetem Volumersatz ergaben im Durchschnitt 21·4 % CO₂, 42·9 % O₂ und 35·7 % N₂; in einer späteren Arbeit werden 10·6 % CO₂ und 22 % O₂ angegeben. Die durch Berechnung bestimmte Zusammensetzung des tatsächlich in den ersten Stunden nach der Punktion in die Blase einströmenden Ersatzgases soll einen Gehalt von 83-85 % CO₂ und 15 % O₂ besitzen (Jacobs, 1932). Die Kohlensäure verschwindet nach vollendetem Volumersatz relativ schnell wieder durch Diffusion aus der Blase, der Sauerstoff bleibt wegen der sehr geringen Durchlässigkeit der

Blasenwandung für dieses Gas in der Blase. Erst im Verlauf von Wochen diffundiert auch der Sauerstoff nach aussen, dafür tritt Stickstoff ein.

Auch Meesters & Nagel (1934) haben den Kohlensäuregehalt des nach einer Druckerhöhung in die Blase sezernierten Gases beim Flussbarsch zu bestimmen versucht. Unter der durch Versuche gestützten Annahme, dass in der Schwimmbase keine Kohlensäure mehr vorhanden ist, wenn im Unterdruckversuch die zuerst schnell verlaufende Resorption langsam geworden ist, wurde das Volumen einer derartig Kohlensäure frei gemachten Schwimmbase mit einer von Jacobs angegebenen Methode gemessen, die Gassekretion durch Erhöhung des auf den Fischen lastenden Druckes angeregt und durch fortlaufende Volummessung des ganzen Fisches in einem allseitig geschlossenen Kasten genau gemessen. Nach 1–2 Stunden wurde der Kohlensäuregehalt einer aus der Schwimmbase entnommenen Gasprobe bestimmt, der 1.9–7.6 %, im Mittel 4.6 % betrug. Aus der sezernierten Gasmenge von im Mittel 170 cmm. ergab sich ein mittlerer Kohlensäuregehalt des Ersatzgases von 86 %.

Bei langsam vor sich gehender Gassekretion kann dagegen die Zunahme des Kohlensäuregehaltes der Blasengase gegenüber der starken Zunahme des Sauerstoffgehaltes auch beim Barsch völlig zurücktreten oder garnicht vorhanden sein (z. B. 1.1 % CO₂ und 8.7 % O₂ vor der Punktion gegen 1.6 % CO₂ und 44.6 % O₂ 7 Tage nach der Punktion bei einem ca. 72 % Ersatz der punktierten Blasengase (Jacobs, 1932)).

Die Tatsache, dass früher von anderen Autoren bei den Blasengasanalysen nach Punktionen diese Zunahmen des Kohlensäuregehaltes, jedenfalls in den von Jacobs beim Barsch gefundenen Ausmassen nicht beobachtet wurden—nach Bohr (1894) z. B. stieg bei *Gadus callarias* nach Punktion der Kohlensäuregehalt von im Mittel 1.2 auf 2.4 %, während der Sauerstoffgehalt von 19.1 auf 39.3 % sich erhöhte—wird darauf zurückgeführt, dass diese Bestimmungen erst längere Zeit nach vollständigem Ersatz der Gase ausgeführt wurden, als die Kohlensäure schon wieder herausdiffundiert war. Dabei konnte z. B. Hall (1924) bei Barschen nach 10-stündiger Druckerhöhung z. T. überhaupt kein Zunahme des Kohlensäuregehaltes und 12 Stunden nach einer Punktion nur geringfügige Zunahmen, im Durchschnitt von 0.64 % auf 2.1 %, beobachten, zu einer Zeit also, wo, wenn überhaupt schon, der Volumersatz höchstens gerade vollendet ist. Und Jacobs (1934) selbst musste bei Ausführung derselben Versuche an einigen Seewasserphysoklisten feststellen, dass der Barsch bezüglich der Kohlensäure durchaus eine gewisse Sonderstellung einzunehmen scheint, trotz im wesentlichen gleichen Baues der Gasdrüsen und Wundernetze. So änderte sich die Zusammensetzung der Blasengase nach der der Punktion bis zum vollkommenen Volumersatz

	% CO ₂		% O ₂		% CO ₂		% O ₂	
bei <i>Gadus morrh.</i>	von	0.5	und	19	auf	2.8	und	48
„ <i>Crenilabrus pav.</i>	„	1.7	„	23.4	„	1.8	„	45.2
„ <i>Box salpa</i>	„	1.1	„	13.3	„	4.3	„	33.8
„ <i>Labrus fest.</i>	„	0.7	„	19.6	„	1.5	„	43.7
„ <i>Sargus rond.</i>	„	0.5	„	26.0	„	2.6	„	59.4

Dagegen wurde auch bei einigen untersuchten Cypriniden und besonders beim Hecht eine beträchtliche Zunahme des Kohlensäuregehaltes festgestellt (Jacobs, 1934).

In eigenen (unveröffentlichten) Untersuchungen konnte ich bei den verschiedensten Fischarten (und zwar bei folgenden Physoklisten: *Serranus cabrilla*, *Smaris alcedo*, *Cantharus lineatus*, *Pagellus erythr.*, *Crenilabrus pavo*) während der verschiedensten Zeiten des Gasersatzes und kurze Zeit nach vollendetem Volumersatz (6–24 Stunden nach der Punktion) z. T. überhaupt keine, in der Regel nur geringfügige Zunahmen des Kohlensäuregehaltes beobachten. Trotz Steigerungen des Sauerstoffgehaltes auf weit über 80 % (bis zu 93 % nach einer 2-maligen Punktion) nahm der Kohlensäuregehalt im Durchschnitt nur von 1.4 % auf 3.1 % zu; dabei waren gerade bei den sehr schnell sezernierenden *Serranus*-Arten die geringsten Zunahmen des Kohlensäuregehaltes feststellbar. Selbst in den oben schon erwähnten Versuchen (v. Ledebur, 1929), in denen das hauptsächliche Resorptionsorgan der Fische (*Serranus*) operativ ausgeschaltet und die Fische infolgedessen zu leicht geworden waren, wurden nach Entleerung der Schwimmblasengase nur geringfügige Zunahmen des Kohlensäuregehaltes bis zu höchstens 3 % beobachtet, während der Sauerstoffgehalt bis auf 94 % ansteigen konnte (die Analysen erfolgten immer an dem Tage, an dem die Fische zu leicht geworden waren). Und auch bei trommel-süchtigen, aus grösserer Meerestiefe kommenden Fischen konnte kein besonders hoher Kohlensäure-Prozentgehalt (0–3 %, im Mittel 2.2 %) gefunden werden (v. Ledebur, 1936), obwohl doch die Gasdrüse bei diesen Fischen wegen der erhöhten Gasdiffusion infolge des hohen Druckes der Blasengase in der Tiefe dauernd in verstärktem Masse tätig gewesen sein muss (s. oben).

Es ist also festzustellen, dass beim Volumersatz nach einer Blasenpunktion oder einer Druckerhöhung zwar sehr häufig eine Zunahme des Kohlensäuregehaltes auftritt, die allerdings meist, ausser in den Versuchen von Jacobs beim Barsch und beim Hecht nur geringfügig ist, und im Mittel 2–5 % in der Regel nicht überschreitet. Schon dieses mitunter völlige oder fast völlige Fehlen einer Zunahme des Kohlensäuregehaltes bei einer ganzen Reihe von Fischen trotz ebenfalls vorhandenen und ähnlich gebauten Wundernetzen und Gasdrüsen und ebenso schneller Gassekretion in der Blase wie beim Barsch spricht kaum für eine allgemeine und alleinige Gültigkeit der Jacobs'schen Arbeitshypothese.

Es ist daher zu überlegen, ob diesem (wohl mit grosser Wahrscheinlichkeit vorhandenem) Mechanismus der Erhöhung des Sauerstoffdruckes durch Zunahme des Kohlensäurepartialdruckes wirklich für den Vorgang der Gasausscheidung in die Blase die ausschlaggebende Rolle zukommt, die ihm von diesen Autoren gegeben wird. Dieser Vorgang könnte ja auch nur, wie es Haldane annimmt, und was mir nach den bis jetzt vorliegenden Versuchsergebnissen am wahrscheinlichsten erscheint, die vorbereitende Aufgabe besitzen, den Sauerstoffdruck in den Gasdrüsenkapillaren zu erhöhen, damit ihn diese Zellen leichter aufnehmen können, um (bei saurer Reaktion) aus ihm jene bis jetzt allerdings noch hypothetische Sekrets substanz zu bilden, bei deren Zerfall Sauerstoff und wohl auch Stickstoff gegen jeden Druck frei wird. Die Kohlensäure könnte als Endprodukt bei den Stoffwechselvorgängen

in der Gasdrüse selbst entstehen. Die Aufgabe des Wundernetzes könnte mit dieser Erleichterung der Dissoziation des Oxyhämoglobins erschöpft sein, es ist aber auch möglich, dass noch irgend eine andere chemische Substanz, die die Gasdrüsen für die Gassekretion benötigen, durch das Wundernetz festgehalten und an einem Abtransport durch das venöse Blut verhindert werden soll. Es muss ferner darauf hingewiesen werden, dass bis jetzt experimentell nur bei niedrigen Sauerstoffspannungen ein Einfluss der Kohlensäure auf die Erhöhung des Sauerstoffdruckes im Blute untersucht und festgestellt wurde (Bohr, *et al.*, 1904; Krogh & Leitch, 1919; Root, 1931). Ob durch Erhöhung des Kohlensäurepartialdruckes oder Säuerung des Blutes (was ja auch nur bis zu einem gewissen Grade stattfinden kann) eine Erhöhung des Sauerstoffdruckes im Blute auf mehrere Atmosphären erzielt werden kann, die auch bei in grösserer Tiefe lebenden Fischen ausreicht, um Sauerstoff auf dem Diffusionswege in die Blase zu befördern, müsste experimentell erst noch geklärt werden.

Es ist schliesslich bei der beobachteten Zunahme des Kohlensäuregehaltes der Blasengase bei der Gasausscheidung daran zu denken, dass infolge der sehr leichten Diffusionsmöglichkeit (s. oben) die Kohlensäure auch aus andern Gründen in die Blase gelangen kann. So besteht die Möglichkeit, dass z. B. die bei den gesteigerten Stoffwechselvorgängen in der Gasdrüse gebildete und wegen der sauren Reaktion derselben nicht gebundene Kohlensäure in die Blase hereindiffundiert. In welchem Masse und wie schnell eine derartige Kohlensäurediffusion stattfinden kann, zeigen z. B. Versuche von Hall: schon in den ersten Minuten nach dem Hereinsetzen von Fischen (Barsche) in Kohlensäurehaltiges Wasser wurde eine vorübergehende Volumzunahme der Blasengase der Tiere beobachtet (nach Hall ein automatischer Mechanismus, um Fische aus schlechtem Wasser an die Oberfläche in günstigeres Wasser zu bringen). Erhöhung des Kohlensäuregehaltes des Wassers bewirkt ebenso wie eine Erniedrigung des Sauerstoffgehaltes des Wassers eine Zunahme der Kohlensäure der Blasengase.

Neuerdings sind dann auch Beobachtungen über Veränderungen in der Gasdrüse von trommelsüchtigen Fischen (*Serranus cabr.*) veröffentlicht worden (v. Ledeber, 1936), die dagegen zu sprechen scheinen, dass schon in dem Blut der Wundernetz- und Gasdrüsenkapillaren eine derartige Erhöhung der Gasspannungen stattfindet, die zu einer Diffusion von Sauerstoff in die Schwimmbase führt. Diese Veränderung besteht in einem Aufschäumen der Gasdrüse, wenn der auf diesen Fischen lastende Wasserdruck (ca. 100 m. Wasser) plötzlich durch Heraufziehen an die Wasseroberfläche entfernt wird, und die Erscheinungen der Trommelsucht auftreten. Die genaue mikroskopische Untersuchung ergab, dass die Gase nicht in den Blutkapillaren des Wundernetzes oder der Gasdrüse frei geworden waren, sondern an der Oberfläche der Gasdrüse und zwischen den Gasdrüsenzellen. Es hatte vielmehr den Anschein, wofür auch das Vorhandensein sicher unveränderter Drüsenzellen sprach, als ob ein von diesen Zellen gebildetes Sekret bei der Druckentlastung aufgeschäumt wäre.

Von viel grösserer Bedeutung ist aber, dass mit dieser Theorie, die, wie oben gezeigt wurde, bei den verschiedensten Fischen sicher vorhandene aktive Aus-

scheidung von Stickstoff in die Schwimmblase entgegen dem Diffusionsgefälle, dem Verständnis nicht näher gebracht wird. Powers (1932) hat diesem Bedürfnis Rechnung getragen, indem er beide soeben erwähnten Erklärungsmöglichkeiten zu einer Theorie zusammengefasst hat. Auch Powers nimmt eine in den Wundernetzen sich immer weiter steigende Erhöhung des Sauerstoffdruckes in dem Blute, das die Gasdrüse versorgt, durch Erhöhung der Kohlensäurespannung an. Die Abgabe der Gase soll jedoch nicht direkt auf dem Diffusionswege in die Blase erfolgen, sondern schon in den Gasdrüsenzellen soll es zur Bildung von Gasbläschen kommen. Dies könnte auftreten, wenn der Gesamtdruck der gelösten Gase, also besonders des Sauerstoffs, grösser als der hydrostatische Druck (Wassersäule über dem Fisch) plus dem atmosphärischen Druck wird. In diesen Bläschen werden alle in den Geweben gelösten Gase, also auch Stickstoff entsprechend ihrem Partialdruck hineindiffundieren und auf diese Weise mit in die Blase gelangen können. Bei der gleichzeitigen Annahme einer dauernden und sofort einsetzenden Rückresorption des Sauerstoffs und einer Undurchlässigkeit der übrigen Blasenwandung für den Stickstoff, könnte damit vielleicht eine Stickstoffausscheidung entgegen dem Diffusionsgefälle erklärt werden. Aber auch dies führt zu keiner endgültigen Erklärung, da, wie wir oben gesehen haben, in Wirklichkeit keine Gasbläschen in den Gasdrüsenzellen nachweisbar sind.

VI. SCHLUSSBEMERKUNG

So wäre zum Schluss wohl zusammenfassend zu sagen, dass wir bis jetzt über die bei der Gassekretion stattfindenden Prozesse trotz vieler Einzeltatsachen eindeutig noch nicht Bescheid wissen, und dass es erst nach weiteren experimentellen Untersuchungen möglich sein wird, eine Entscheidung darüber zu treffen, ob und wie weit die bis jetzt aufgestellten Theorien zur Erklärung anwendbar sind.

VII. ZUSAMMENFASSUNG

1. Nach Besprechung der Zusammensetzung der Schwimmblasengase bei verschiedenen Fischarten werden die physikalischen Verhältnisse erörtert, unter denen sich einmal die im Wasser gelösten Gase und ferner die Schwimmblasengase befinden. Auf Grund dieser Tatsachen muss eine entgegen dem Diffusionsgefälle stattfindende Ausscheidung von Gasen, und zwar von Sauerstoff und von Stickstoff in der Schwimmblase, jedenfalls bei den meisten Fischen, angenommen werden.

2. Eine derartige Gasausscheidung erfolgt immer bei einer Erhöhung des spezifischen Gewichtes, eine Resorption von Gasen bei Verringerung des spezifischen Gewichtes des Fisches; dabei sind in der Hauptsache Änderungen des Sauerstoffgehaltes der Blasengase zu beobachten.

3. Die Gasausscheidung steht unter Nerveneinfluss, und zwar verlaufen im Vagus die die Gasausscheidung fördernden Impulse, im Sympathicus die sie hemmenden Impulse. Die Einstellung des spezifischen Gewichtes, durch Gasausscheidung oder Gasresorption, erfolgt reflektorisch, wobei als auslösender Reiz eine Bewegung oder Abbiegung von Flossen funktioniert.

4. Physostomen, und auch Jungfische einiger Physoklisten, die eine Zeit lang einen offenen Luftgang besitzen, können durch Verschlucken atmosphärischer Luft an der Wasseroberfläche die Schwimmlase mit Gas füllen. Daneben findet aber bei vielen Physostomen eine, nur meist viel trägere Gasausscheidung wie bei den Physoklisten statt.

5. Als Ausscheidungsort für die Blasengase kommt die sogenannte Gasdrüse in Betracht, auf deren histologischen Aufbau und Blutversorgung (Wundernetze) genauer eingegangen wird. Versuchsergebnisse sprechen dafür, dass hier eine dauernde Gasausscheidung stattfindet, zu der im Bedarfsfalle eine zusätzliche, nervös regulierte Tätigkeit der Gasdrüse hinzukommt.

6. Die Gasresorption erfolgt in dem "hinteren Gefässorgan" (Oval), wobei hauptsächlich Diffusionsvorgänge eine Rolle spielen. Aber auch andere Teile der Blasenwandung sind für Gase, ganz besonders auch für Kohlensäure durchlässig.

7. Zum Schluss werden die verschiedenen Theorien, die zur Erklärung des Vorganges der Gassekretion aufgestellt worden sind, kritisch besprochen. Während von einigen Forschern angenommen wird, dass tatsächlich in den Gasdrüsenzellen, entsprechend ihrer Lage als ein die Blase auskleidendes Epithel die Gassekretion stattfindet, wird besonders neuerdings der Gasdrüse nur die Fähigkeit der Bildung eines besonderen Sekretes zugeschrieben, das in die Blutkapillaren abgeschieden wird und in diesen, hauptsächlich in den Wundernetzen, eine Erhöhung der Gasspannung hervorrufen soll. Dies Sekret soll sauren Charakter besitzen, die Kohlensäurespannung des Blutes erhöhen, die wiederum zu einer Erhöhung der Sauerstoffspannung des Blutes führt. In den Wundernetzen soll durch Diffusion und mehrmalige Wiederholung dieses Vorganges eine Erhöhung der Sauerstoffspannung im Blute stattfinden, bis schliesslich durch Diffusion Sauerstoff in die Schwimmlase gelangt. Es werden Bedenken und Versuchsergebnisse angeführt, die gegen das *alleinige* Vorhandensein dieses zuletzt beschriebenen Mechanismus sprechen, und die es wahrscheinlich machen, dass dieser Vorgang der Zunahme des Sauerstoffdruckes durch Erhöhung des Kohlensäurepartialdruckes nur eine vorbereitende Aufgabe besitzt, um den Gasdrüsenzellen die Bildung ihres Sekretes zu erleichtern, bei dessen Zerfall Sauerstoff und Stickstoff in der Schwimmlase gegen jeden Druck frei werden.

VIII. SUMMARY

1. The composition of the gases in the swim-bladders of different fishes is first considered, after which the physical conditions are discussed under which gases are found when dissolved in water and when present in swim-bladders. It follows from the data that there must be a liberation of gases, particularly of oxygen and nitrogen, into the swim-bladder against the diffusion gradient.

2. Gas liberation always occurs when the specific weight of the fish rises, while gas is resorbed when the specific weight falls. On these occasions it is especially the oxygen content of the bladder which is altered.

3. Gas liberation is under nervous control. The nerve impulses causing an increase in gas liberation pass through the vagus and the inhibitory impulses pass through the sympathetic. The establishment of the specific weight, whether through gas liberation or resorption is a reflex, of which the stimulus is a movement or flexion of fins.

4. Physostomids, and also the young of some physoclystids which have an open pneumatic duct for a certain time, can fill the swim-bladder with gas by swallowing air at the water surface. But, in addition to this, many physostomids also produce gas, though to a much less degree than the physoclystids.

5. The so-called gas gland is the seat of gas liberation. The histology of the gas gland and its blood supply (rete mirabile) are dealt with. Experimental results indicate that a continuous liberation of gas takes place here and that in case of need there is additional activity of the gas gland controlled by the nervous system.

6. Gas resorption takes place in the "posterior gas organ" (oval), mainly by diffusion. But other parts of the swim-bladder wall are permeable to gases, particularly to carbon dioxide.

7. In conclusion the various theories put forward to explain gas secretion are critically discussed. Some workers assume that gas secretion actually takes place in the cells of the gas gland, forming, as they do, an epithelium lining the bladder. But more recently it has been maintained that the gas gland merely produces a particular secretion which passes into the blood capillaries, where it causes an increase in gas tension, particularly in the rete mirabile. This secretion is said to have an acid character, raising the carbonic acid tension and thus causing a rise in oxygen tension in the blood. In the rete mirabile, diffusion and repetitions of this process are said to bring about a rise in the oxygen tension of the blood until eventually oxygen passes by diffusion into the bladder. But there are arguments and experimental data which speak against the phenomena just described as being the sole mechanism, and which make it probable that this process of increase of oxygen pressure through a rise in carbon dioxide partial pressure has only a preparatory role, facilitating the process of secretion by the gas gland cells, through the breakdown of which oxygen and nitrogen are liberated against pressure into the swim-bladder.

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THE SPECIFICITY AND COLLABORATION OF DIGESTIVE ENZYMES IN METAZOA

By H. J. VONK

(Laboratory of Comparative Physiology, University of Utrecht, Holland)

(Received 17 March 1936)

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I. INTRODUCTION

MODERN work on enzymes is carried out by exact quantitative methods for the determination of the substrates or scission products as well as for pH measurement, and care is taken to keep constant the hydrogen-ion concentration and temperature in the experiments. Only such work will be considered in this review. In the older investigations the reaction of the medium in which the enzymes worked was simply indicated as neutral and slightly acid or alkaline. In many cases the presence of an enzyme could only be detected by the disappearance of a solid substrate or by some qualitative change of a liquid one. Although this mode of work sometimes allowed of a rough estimation of the strength of the enzyme solution, it was impossible to follow the process by quantitative methods. Since the introduction by Sørensen (1909) of the symbol *pH*, the methods for the determination of this exponent and the means for keeping it constant have been brought to a high degree of perfection. Since that time also many excellent methods have been developed for following the course of proteolysis. They consist either in the determination of amino acids in media of formol (Sørensen, 1908 *a, b*), alcohol (Willstätter & Waldschmidt-Leitz, 1921 *a*) and acetone (Linderström-Lang, 1927) or by Van Slyke's gasometric method (1911), or in measurement of the change of physical properties, such as viscosity (Ringer, 1923) or electrical conductivity (Northrop, 1922) of the digestive fluid. Accurate measurements of amylolytic and lipatic activity have been possible for

a long time by means of polarimetry or sugar titrations and titration of fatty acids. However, the quantities of the juices or extracts dealt with in comparative physiology are mostly so small that it has sometimes been necessary to wait for the development of suitable micro-variations before these methods could be applied in this field. For the determination of amino acids a micro-method has been worked out by Grassmann & Heyde (1929 *a*) and by Linderström-Lang & Holter (1931), for monoses by Hagedorn-Jensen (1922 *a, b*). A good review of all the methods alluded to here and of others is given by Rona (1926) in his book *Fermentmethoden* (2nd ed. 1931).

Researches where such exact methods have not been used will only be alluded to in so far as is necessary to make the problems clear.

Besides limiting the article to a review of methodical modern papers, it has also been limited for another reason. Not all the work which has been done with modern methods is at present suitable for generalization. It seems to the author that more and better results would have been reached if more elaborate work had been done on a smaller number of species, representing the big phyla, instead of investigating, in most cases superficially, a great number of animals.

Tables and references showing the occurrence of enzymes in all the species of invertebrates which have been investigated are found in Oppenheimer's *Fermente* (I and II, 1925-6), in Weinland (1909), Schulz (1925), Jordan (1913), v. Fürth (1903), Yonge (1931) and Krüger (1933, 1934). It is proposed here to deal only with the recent literature which may lead to satisfactory answers to the following theoretical questions.

(1) Is it possible to classify all the enzymes found in Metazoa according to the better known types of enzymes of mammals and plants, or will it be necessary to create new types of ferments for lower vertebrates and invertebrates? This is the problem of the specificity or identity of enzymes.

(2) Are there differences in localization of the enzymes in the different groups of animals, and can these differences be related to the organization of the animal?

In attempting an answer to the above questions, it is necessary to take into account many facts which are known from physiological chemistry.

II. SPECIFICITY OF VERTEBRATE AND INVERTEBRATE ENZYMES

(1) *Means for distinguishing enzymatic specificity*

The first researches on the agents which we now call enzymes were carried out at the beginning of the nineteenth century (Kirschhoff, 1815; Liebig & Wöhler, 1837; Schwann, 1836; Payen & Persoz, 1833, 1834). From these researches it already appeared that one of the most remarkable properties of this group of agents is the specificity in their power of accelerating chemical reactions. Later on, the various enzymes were classified into groups of protein-splitting enzymes (nowadays called proteases), of fat-splitting enzymes (lipases) and of carbohydrate-splitting ferments (carbohydrases). By this last classification there was introduced into the doctrine of enzymes what we now call a group specificity.

The first means for distinction between different digestive enzymes resulted from the ability of a definite juice or extract to cleave certain substrates to the exclusion of others. A favourable circumstance for the development of our knowledge has been that the earlier investigations on enzymes mainly concerned the digestive juices of vertebrates. As the different enzymes in the alimentary canal of these animals are to a high degree localized in different organs, the early investigators had at their disposal a number of enzymes which were to a certain degree pure in their natural state, or at least free from considerable amounts of other closely related enzymes. Saliva, which acts only on starch and very slightly on maltose, can only contain what we now call an amylase. However, it had to remain undecided whether in the intestinal extract, which can split both starch and maltose, both of those actions are to be ascribed to one enzyme or to a separate amylase and maltase. The existence of an independent amylase in saliva being known, the latter possibility has been considered the most probable, but a definite proof has not been given. Similarly it has been considered probable that trypsin, lipase and amylase of the pancreas are three separate enzymes, amylase occurring independently in saliva and the two other enzymes, if not separately, at least in other combinations in yeast and seeds. A proof for the independent existence of these enzymes has been given already by Danilewsky (1862) and Cohnheim (1863) by means of precipitation and adsorption. These important results have, however, been neglected. Recently Willstätter and his co-workers have succeeded in separating the three enzymes again by adsorption methods (Willstätter & Waldschmidt-Leitz, 1923 *a*; Willstätter *et al.* 1923 *b*).¹

But even before evidence for the independent existence of all the now well-known enzymes of the vertebrate digestive tract was gathered from their localization and mode of working on different substrates another means of distinguishing between related enzymes seemed to be possible. This means is based on the necessity of a pronounced acid reaction for the action of the stomach protease (pepsin), and of a slightly alkaline reaction of the medium in which the pancreatic protease (trypsin) can show its activity. As the lowest scission products for the first enzyme are albumoses and peptones, and as the second attacks, besides proteins, also albumoses and peptones with the formation of a small amount of amino acids, there has been no doubt that the two enzymes are totally different. Thus it has been possible to distinguish between pepsin and trypsin by varying the reaction of the medium. This last means of identification was largely adopted in comparative physiology. A protease working only in acid medium was concluded to be a pepsin, while that acting only in alkaline medium must be a trypsin. In particular Krukenberg (1878) made use of this reasoning (see further the quotations of his work in Von Fürth, 1903; Jordan, 1913; Biedermann, 1911). Von Fürth (1903, p. 225) had already warned workers against uncritical introduction of definitions like pepsin and trypsin from vertebrate physiology into comparative physiology. The question is also discussed by Jordan (1907). It is to be noted, moreover, that in the time of Krukenberg the concentration of hydrogen ions (the real acidity) could not be

¹ When there are several collaborating authors, the first only is usually cited in the text.

determined. The acidity or alkalinity was expressed in titration values (potential acidity), which (owing to the buffer action of proteins, etc.) is no measure for the real acidity.

After Sørensen (1909, 1912) had introduced convenient methods for measuring the hydrogen-ion concentration (generally expressed as pH) in physiological chemistry, and after, in the same period, it became possible to regulate the pH in much finer gradations and to keep it constant by means of buffer mixtures, it was found that all enzymatic reactions show a dependence on the pH , which could be easily expressed graphically, plotting pH against activity. Much work has been done in determining these pH -activity curves (or pH -optimum curves) which have been considered as more or less characteristic for each separate enzyme. The

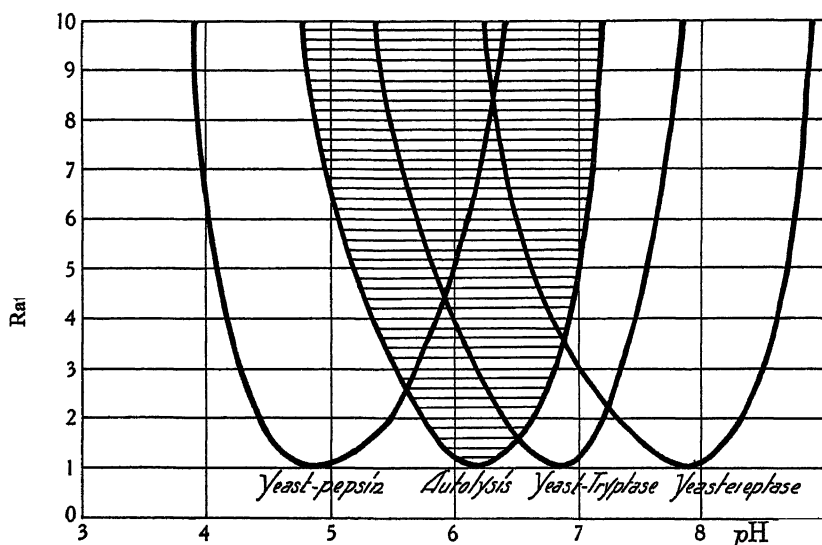


Fig. 1. pH optima of yeast proteases, after Dernby (1917). The optimum for autolysis is the result of the combined actions of the three enzymes present.

amylases of mammals (from saliva and pancreatic extracts) show an optimal activity at pH between 6 and 6.8, whereas for vegetable amylases (from malt and yeast) the optimal values are situated at pH 4-5 (see Oppenheimer, I, 1925-6, pp. 691, 692). Dernby (1917) carried out an analysis of the yeast proteases, and from the three optima at pH 4.8, 7.0, and 7.8 concluded that there are three separate proteases in yeast (pepsinase, tryptase and peptidase). For the autolysis he found an optimum at 6.2, which he ascribes to the joint activity of the two proteases and the peptidase (see Fig. 1).

These results indicated methods for identifying the different enzymes of invertebrates and fishes. Attempts in this direction have been made for coelenterates by Bodansky & Rose (1922), for the enzymes of fishes by Vonk (1927), for the proteases of the crayfish (*Potamobius fluviatilis*) by Shinoda (1928), for the amylase and maltase of the same animal by Wiersma & Van der Veen (1928), for

different enzymes of several marine animals by Yonge (1923, 1924, 1925 *a, b*, 1926, 1930), for different enzymes of the crayfish, the snail (*Helix pomatia*) and many other invertebrates by Krüger (1926, 1929) and Krüger & Graetz (1927 *a, b*, 1928). Although this method has given valuable preliminary results, it was found later on to be incompatible with some of the general properties of enzymes, which have been recently discovered.

From the physico-chemical side it has been discovered that the *pH* optimum is not at all constant for any definite enzyme working on different substrates. In some cases, e.g. pepsin and trypsin, for each substrate a different *pH* optimum has been found. The table on p. 909 of Oppenheimer's *Die Fermente und ihre Wirkungen*, II (1925-6) gives fourteen different optima for the action of trypsin on seven different substrates. Some of these optima are shown in Fig. 2. These

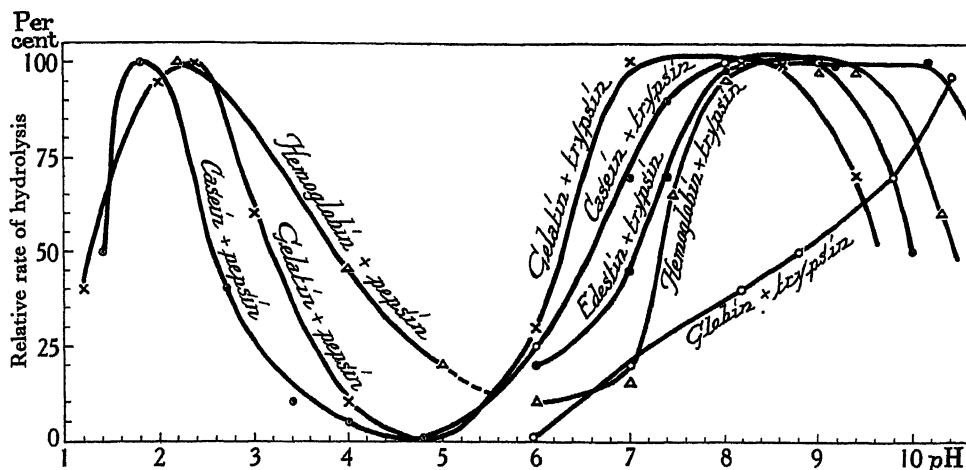


Fig. 2. *pH* optima of pepsin and trypsin in four different substrates, after Northrop (1923). This figure also illustrates Northrop's conception that pepsin can act only on protein cations, trypsin on protein anions. The optima of trypsin especially are situated at the highest *pH* for those substrates, which have the highest isoelectric points (compare p. 255).

differences are partly due to the different durations, temperatures, and buffers which these investigators used in their determinations. To a certain degree these controversies could be settled by repeating the experiments and comparing with invertebrate enzymes under exactly the same conditions.

From the side of organic chemistry the work of Willstätter and his collaborators has largely contributed to solve the problems of specificity. Willstätter (1922, 1926 *a*, 1927, 1928) introduced the method of adsorption in the research on enzymes. Using several adsorbents like aluminium hydroxide ("Tonerde"), kaolin, ferric hydroxide in different concentrations and at different *pH*, Willstätter succeeded in freeing the enzymes from impurities and separating them from each other (see also a review by Langenbeck, 1933, p. 490). First the activity of the original solution on different substrates is tested, and the course of the separation and purification is followed by determining the activity of the solution after

each adsorption. After each adsorption the adsorbate is separated from the fluid by centrifuging. If the desired enzyme remains largely in the solution and the unwanted enzymes and impurities are adsorbed, the remaining solution can be used as such. If the desired enzyme, on the contrary, is adsorbed and the unwanted bodies remain in the solution, the enzyme must naturally be set free from the adsorbate. This process, referred to as "elution", is carried out by means of weak electrolyte solutions (e.g. phosphates). It may be necessary, before the final separation is attained, to repeat the adsorption with the same adsorbent, to vary the acidity of the medium or to change the adsorbent. Which combination of adsorptions will lead to the goal must be empirically tried for each case.¹

When an enzyme is purified in this way, its properties may change. For instance, the *pH* optimum can be shifted considerably, the adsorption properties may change, and the stability of the enzyme always decreases. The latter seems to be due to the removal of colloids. On this and other arguments, Willstätter bases his view that the active part of the enzyme is carried by a colloidal carrier (Willstätter, 1922). In purifying the enzymes part of the carriers is removed or deteriorates, the active groups being probably heaped up on a smaller amount of carriers. This instability ultimately limits further purification.

The only property of the enzyme which remains constant during the purification is its specificity for definite substrates (Willstätter, 1926 *b*, p. 941).

As the *pH* optimum depends largely on the state of purity of the enzyme and on the kind of substrate used, it is clear that the determination of the *pH* optima is not a very safe means for the identification of enzymes. The best method for this purpose so far known is that of adsorption described above. The application of this method is all the more necessary in the case of the digestive juices of invertebrates, where in most cases all the enzymes occur together in one division of the alimentary canal. On the other hand the smallness of invertebrate organs, and the limited amount of juice available, present serious difficulties in the application of these methods. For a thorough analysis it is therefore advisable to choose big organisms for such investigations.

Continued investigations of Willstätter have informed us that part of the enzymes can be liberated easily from the cells (lyo-enzymes), the other part being firmly anchored to several cell components (desmo-enzymes). The combination of enzyme and cell component is called simplex and the same expression is used for the combination of active group and colloidal carrier of the enzyme (for literature see Willstätter and Rohdewald, 1934).

It has long been discussed whether only the active group (agon²) or also the colloidal carrier (pheron²) is responsible for the *specificity* of the enzyme. Researches of Bamann & Laeverenz (1930 *a*, *b*, 1934) had made it probable that the

¹ Several enzymes (pepsin, trypsin, urease) have been isolated in a "crystalline" state (Northrop & Sumner; Northrop, *Biol. Rev.* 1935). These procedures have not yet been applied in comparative physiology. Discussion of these results in the literature has made it rather doubtful whether the crystalline products are the true enzymes (consult *J. gen. Physiol.* 1930-6). The results have not changed our knowledge of the specificity derived from adsorption experiments.

² Thus termed by Kraut (1934 *b*).

specificity of lipases and esterases could be influenced by substances which only cause a change of the carrier. Kraut (1934 *b*) has succeeded in confirming these facts by ingenious experiments, which allowed him to change pancreas lipase into liver esterase and the reverse. Pancreas lipase is rather unstable and its lability can be reinforced artificially. Liver esterase is stable. If Willstätter's conception of enzymatic composition be true, the pancreas lipase must be poor in pheron and (being active) rich in agon, whereas the liver esterase must be rich in pheron and by artificial decrease of its activity can be deprived partly of its agon. Kraut now asked himself whether between simplex, agon and pheron an equilibrium reaction could exist: $\text{simplex} \rightleftharpoons \text{agon} + \text{pheron}$, and expected that the dissociation constant $K = \frac{[\text{pheron}] \times [\text{agon}]}{[\text{simplex}]}$ might be rather large. In that case an excess of pheron would be necessary to stabilize an enzyme. Thus it would be possible to stabilize labile pancreas lipase by adding liver esterase, which is rich in pheron and poor in agon. In performing this experiment a stable enzyme resulted, which had more the properties of a liver esterase than of a pancreas lipase. However, the active groups were provided by the pancreas lipase, and thus the conclusion must be that (at least in this case) the specificity of the enzyme for a special substrate is due to the properties of the pheron. Probably the agon is the same for pancreas lipase and liver esterase. This was already indicated by an experiment of Virtanen & Suomalainen (1933), who, on injecting pancreas lipase into the blood, found an increase of esterase in the liver and not of lipase in the pancreas. These results are a strong argument for Willstätter's conception of the composition of enzymes. Moreover, the results with the enzymes of biological oxidation (for literature see Kraut, 1934 *b*) are in perfect agreement with this view.

(2) *Specificity of proteases*

Proteins are built up of amino acids by means of the peptide linkage. It was Emil Fischer's conception that the protein molecules consisted of long chains of such acids. Later on, under the influence of Röntgen spectrography and additional chemical facts, many authors assumed that the protein molecules ("Grundkörper") contained a small number of amino acids and were of a cyclic structure. These molecules would combine by means of micellar forces to larger aggregates.

The latest data, however, gathered especially from enzymatic hydrolysis of proteins, have caused a return to Fischer's conception of long chains and excluded the presence of a large number of cyclic bonds. Later results of Röntgen spectrography also agree with this conception. For details and literature, reviews by Sørensen (1930) and Linderström-Lang (1933 *a*) may be consulted.

Before considering the work that has been done on higher and lower animals, it is necessary to give a short account of the results which were arrived at with proteases in general. Most of the work on this subject concerns the proteases of mammals and plants, and has been carried out by Willstätter, Waldschmidt-Leitz, Grassmann and their collaborators (see Waldschmidt-Leitz, 1929 *a*, and the review by Linderström-Lang, 1933 *a*).

According to these authors there are five main types of proteases, viz. the digestive proteases, the tissue proteases, the yeast proteases, the proteases of papain and those of the white blood corpuscles. The digestive proteases will be considered first. They are enumerated in Table I. The left side of the table is a review of the enzymes and their properties before the work of Willstätter *et al.* had started. The right side is the expression of our present knowledge. The table is to be read from the inner towards the outer columns. In this way the old and new nomenclatures can easily be compared in the two middle columns.¹

Table I. *Digestive proteases of the mammalian intestinal tract*

Acti- vator	Cleavage products	Substrate	Enzyme, old name	Enzyme, new name	Substrate	Cleavage products	Acti- vator
—	Albumoses and pep- tones	Genuine proteins	Pepsin	Pepsin	Genuine proteins, histones	Albumoses and pep- tones	—
Entero- kinase	Albumoses, peptones and amino acids	Genuine proteins, albumoses	Trypsin	1. Tryptic proteinase	Genuine proteins	Albumoses, peptones and poly- peptides	Entero- kinase
—	—	—	—	2. Carboxy- polypepti- dase	Poly- peptides	Dipeptides and amino acids	Entero- kinase
—	—	—	—	3. Prot- aminase	Prot- amines	Polypep- tides and amino acids	—
—	Peptides and amino acids	Albumoses and pep- tones	Erepsin	1. Aminopoly- peptidase	Poly- peptides	Dipeptides and amino acids	—
—	—	—	—	2. Dipepti- dase	Dipep- tides	Amino acids	—

As is clear from Table I, the position of pepsin, acting on genuine proteins and producing albumoses and peptones as cleavage products, has remained unaltered. Trypsin, which was considered to split genuine proteins only, has been found to be composed of two enzymes: (1) true trypsin or tryptic proteinase,² which has the properties of the former "trypsin" (opt. pH 8.2-8.7), and (2) carboxypolypeptidase. This last enzyme is able to split up polypeptides. The result of its action are dipeptides and some single amino acids, as tryptophane, leucin and tyrosin. Carboxypolypeptidase causes the cleavage by attaching itself to the free carboxyl

¹ The pH optimum of pepsin is situated at about 2. For trypsin we have already seen that different optima have been found. Most of these values are situated at about 8. However, Ringer (1921, 1923) found for fibrin an optimum at 11.3 for short duration experiments. Vonk & Heyn (1929b) stated that this high optimum is also present for prolonged experiments and that it exists together with the lower optimum of about 8. The high optimum has, of course, no biological significance. The optima of the other enzymes mentioned in Table I are situated at about 8.

² In modern nomenclature the name proteinase is applied to enzymes splitting genuine proteins. The term proteases is used for the whole group of enzymes splitting proteins and their lower derivatives. The enzymes acting on the derivatives of protein only are called peptidases.

group of the substrate.¹ A certain degree of ionization of this group depending on the structure of the whole substrate is necessary. Its action is reinforced by the presence of enterokinase, but it can also act without this kinase. Aminopolypeptidase also splits up polypeptides, but it does so by attaching itself to the amino group of the substrate. The dipeptidase can split up dipeptides only. It attaches itself to their amino group. Aminopolypeptidase and dipeptidase are the components of the former erepsin. Linderström-Lang (1929 *a, b*, 1930) has concluded from certain experiments that for different dipeptides different dipeptidases must be present. This view has been criticized by Grassmann (1929 *b*). As Linderström-Lang has not isolated these enzymes, this question is still undecided.

Properties and separation of these components of trypsin and erepsin have been described by Grassmann and co-workers for yeast (1927 *a, b*, 1928 *a, b, c*), by Waldschmidt-Leitz and co-workers for mammals (1927 *a, b*, 1928 *a, b, c, d*, 1929 *a, b, c*, 1930 *a, b*, 1931 *a, b*), and by Balls & Köhler (1931 *a, b, c*). The conception that the peptidase of the gut is able to act by attaching itself (probably by means of a CO group) to the amino group of the substrate has been forwarded by Euler & Josephson (1926 *a, b*, 1927, *a, b*). (For complete literature Linderström-Lang (1933 *a*) may be consulted.) Pancreatic extract and pancreatic juice contain a small amount of erepsin together with the trypsin. In extracts of the gut, erepsin is more or less mixed with trypsin, which is adsorbed to the wall of the gut. The latter fact explains the observation of older literature that erepsin, besides attacking peptones, can attack certain genuine proteins like casein (Cohnheim, 1901, 1902 *a, b*).

Moreover, Waldschmidt-Leitz *et al.* (1931 *a*, 1933) have observed that the cleavage of protamines is performed by a separate enzyme, "protaminase", which forms part of the former trypsin. This enzyme is also indicated in Table I. It needs no activation, and cannot be activated by enterokinase.

Pepsin is found only in vertebrates (in the stomach and in the glands of Brünner). A variety of pepsin was assumed to be present in tissue extract (Hedin, 1922), in plant extracts (Vines, 1901, 1903, 1905, 1909) and also in the digestive juice of invertebrates (Krüger & Graetz, 1927 *b*). This conception was based on the fact that the pH optimum of the true proteinase of plants and animal tissues is lower than that of pancreatic trypsin. The existence of a vegetable trypsin and a vegetable erepsin were accepted besides that of the pepsin. These views, however, have proved to be erroneous. Modern research has proved that the compounds of vegetable proteases (*viz.* those of papain and yeast), and of tissue enzymes (with

¹ According to the enzyme-substrate theory advanced by Michaelis, Euler, Waldschmidt-Leitz, Grassmann and others, the enzyme attaches itself to the substrate. By this attachment the properties of the substrate change, so that now it can be hydrolysed by water. For this behaviour of the substrate purely chemical analogies are known. The result is the formation of a combination of enzyme and cleavage product. In this combination the union between enzyme and cleavage product is again unstable, so that the two can dissociate from one another. All these reactions are equilibrium reactions. By this theory, the inhibition of an enzyme reaction on addition of a cleavage product is explained: the latter binds a certain amount of enzyme, which now cannot combine with the substrate. That the binding of a certain polypeptidase is performed by the NH₂ or COOH group of the polypeptide is proved by substitution of these groups. The group of the aminopolypeptidase which binds itself to the NH₂ group is probably a CO group. For literature on the enzyme-substrate theory, Waldschmidt-Leitz (1929 *a*, chap. IV) may be consulted.

a slightly acid optimum), resemble those of the trypsin-erepsin system of the vertebrate digestive tract and that a pepsin is absent. These different plant and tissue proteases are enumerated in Table II, which is taken from Waldschmidt-Leitz (1930 *c*), with the addition of the enzymes of papain. The supposed existence of a tissue proteinase with an optimum on the alkaline side has been excluded. Conclusions of earlier workers in this respect were due to the action of erepsin on the alkaline side, which removed the scission products of the proteinase and so caused a secondary alkaline optimum of the latter. (The question of an invertebrate pepsin will be dealt with below.)

Table II. *Types of proteolytic systems*

Mammalian intestinal tract	Mammalian organs (e.g. spleen)	Yeast cell	Papain	White blood corpuscles
Proteinase (trypsin type)	Proteinase* (papain type)	Proteinase (papain type)	Proteinase	Proteinase (trypsin type) Proteinase (papain type)
Tryptic-carboxypolypeptidase	Katheptic-carboxypolypeptidase	—	Polypeptidase	Carboxypolypeptidase
Aminopolypeptidase	Aminopolypeptidase	Aminopolypeptidase	—	Aminopolypeptidase
Dipeptidase	Dipeptidase	Dipeptidase	Dipeptidase	Dipeptidase

* For this proteinase the name of katepsin has been adopted.

A proteinase (giving rise to the formation of polypeptides), a carboxypolypeptidase, an aminopolypeptidase and a dipeptidase have been found in yeast, papain and animal tissues. The differences between these enzymes and the corresponding digestive enzymes of vertebrates lie chiefly in the activation properties.

Among the tissue enzymes (mainly those of the spleen), occur a proteinase (called lieno-protease or katepsin, opt. *pH* 4, Waldschmidt-Leitz, 1927 *c*), and a carboxypolypeptidase (opt. *pH* 4.2). These are naturally activated by a "zookinase", which can be replaced by hydrocyanic acid, hydrogen sulphide and "phytokinase", but not by enterokinase. The specificity is the same as for tryptic proteinase and carboxypolypeptidase. Further, an aminopolypeptidase (opt. *pH* 8) and a dipeptidase (opt. *pH* 8) have been found (Waldschmidt-Leitz, 1929 *e*, 1930 *c*). Contrary to these observations, Hedin (1930) still maintains the presence of a proteinase with alkaline optimum (*pH* 8.9) in spleen extracts apart from the proteinase with acid optimum. According to this author, he could exclude the action of peptidases in determining this optimum. So the question is still awaiting a final decision.

The enzymatic complex of yeast consists of compounds analogous to those from tissues, with the exception of the carboxypolypeptidase which seems to be lacking in yeast (Grassmann *et al.* 1927 *a, b*, 1928 *a, b, c*). Instead of zookinase, the natural activator here is phytokinase. Later it was proved that phytokinase

and zookinase are both identical with the reduced form of glutathione (Grassmann, 1931, for phytokinase; Waldschmidt-Leitz & Purr, 1931 *b*, for zookinase).

Papain consists of a proteinase, polypeptidase and dipeptidase (Willstätter, 1926 *c*; Ambros & Harteneck, 1929). It has long been known that papain can be activated by hydrocyanic acid, as was especially pointed out by Mendel & Blood (1910). For the action on genuine proteins (giving rise to formation of peptones) this activation is unnecessary. By the activation the specificity of the papain system is extended to lower products, viz. peptones, so that amino acids are formed. Hydrocyanic acid can also activate the carboxypolypeptidase. The analysis of this system is not as complete as that of the systems mentioned before. According to Linderström-Lang (1933 *b*), in the juices which are secreted by insectivorous plants a proteinase is present with an optimum at a rather low *pH* (3.30). This enzyme would occupy an intermediate position between the tissue proteinase and the plant proteinases with acid optimum on the one hand and true pepsin on the other hand. In the white blood corpuscles a tryptic proteinase (like that of the pancreas) and a kathepsin are present. A carboxypolypeptidase, an aminopolypeptidase and a dipeptidase have also been found (Willstätter, 1929 *a*, 1929 *b*, 1930).

Resuming all these results it seems that the protein molecule is nowhere broken down by *one* enzyme, but that several enzymes are necessary. Each of the several enzymes is adapted to a special kind of linkage. The term linkage must be understood here in a general sense. With the same configuration of connecting atoms, it is nevertheless possible to speak of several linkages, because of the influence which is effected by the configuration of the combined or of neighbouring molecules on the *stability* of the linkage.

For the mode of action of proteinases it has been stated by Northrop (1923) that pepsin only acts on protein cations, whereas trypsin is only able to act on protein anions (compare Fig. 2, p. 249). According to Willstätter (1926 *c*) papain would attack proteins in an isoelectric state. Objections to this last view have been made by Vonk (1931 *a*) because of the great influence of buffer mixtures on the position of the isoelectric point of fibrin and other proteins. For solid proteins like fibrin Ringer (1921, 1923) demonstrated that the velocity of digestion was parallel to the swelling of the substrate and that the digestion was influenced by the same factors and in the same way as the swelling. These researches have been confirmed by Vonk & Heyn (1929 *b*).

Concerning the identity of enzymes in the various species of mammals not very much is known.

Waldschmidt-Leitz & Shinoda (1928 *e*) investigated the kinases of different mammals in their action on samples of trypsin of the pig, the cat and the sea lion. Trypsin of the pig was activated by an excess of kinase from ten different mammals to the same amount, and the same was the case for trypsin of the cat by enterokinase of cat, dog and rabbit. But for trypsin of the sea lion rather large differences were found in activation with excess of kinase from different animals, so that the authors could not conclude as to the identity of all mammalian kinases.

The specificity of the proteases in lower vertebrates is, as far as investigated, the same as in higher vertebrates. Vonk (1927, chap. v) was able to purify the pepsin of *Acanthias* in the same way as is possible for mammalian pepsin by Pekelharing's method. The trypsin of *Acanthias* has been purified by the same author by the method of Ringer (Vonk, 1927, chap. v). The pH optima of the pepsins of different vertebrates (pig, pike, *Acanthias*, frog and *Testudo*) are almost identical and situated at about pH 2 (Vonk, 1929 c). Adsorption methods have not yet been applied here. The pepsin of fishes (e.g. *Esox lucius*) seems to be less resistant to temperature than that of mammals (Racokzy, 1913); this result has been confirmed by unpublished experiments in our laboratory. Pjatzitzky (1931) found, in the same way, that frog pepsin is less resistant (chiefly between 55° and 65°) than that of man. It is still uncertain whether these results will have a more general value. Probably this difference in resistance is connected with the colloidal carrier of the enzyme. Racokzy diluted the solutions of *Esox* pepsin with a boiled solution of the mammalian enzyme and *vice versa*. The relative instability of the fish enzyme did not change by this mode of dilution compared with the diluted mammalian enzyme and cannot therefore be due to inorganic or stable matter, but rather to substances more or less closely related to the enzyme, such as colloidal carriers or impurities. However, the specificity of the enzyme is not involved in these differences. Similar results have been reached by Koschtanz (1934) for trypsin of warm- and cold-blooded animals.

As to the work that has been done on invertebrate digestive proteases, it has been pointed out already that the first endeavours to identify these enzymes were chiefly based on determinations of the optimal acidity or alkalinity at which they act. Before Willstätter started his work this was the only possibility, since in invertebrates there is seldom any localization and the digestive enzymes are present in *one* mixture.

Detailed tables of all the enzymes which have been found in different animals are omitted here. Considered in the light of modern research these data are no longer of great value. Compare the reviews cited on p. 246 for such tables.

One of the first modern attempts to study more closely the specificity of the proteases of an invertebrate animal we owe to Shinoda (1928). This author determined for the crayfish (*Astacus*) the pH optima of the action of the juice on several substrates. Shinoda classes his results into three optima: one at about pH 5-6.5 for total proteolysis and peptolysis, one at about pH 8 for the beginning of the cleavage of insoluble substrates and one at pH 9 for dipeptides. At the time that these researches were made, the results were taken to indicate similarity between the enzymes of *Astacus* and those of yeast and plants. (Compare the results of Dernby (1917) described on p. 248.)

Further, Shinoda investigated the action of *Astacus* on connective tissue (catgut and ligamentum nuchae) which in vertebrates is digested only by pepsin. The juice of *Astacus* is able to act on these substrates to a slight extent. Optima are situated at pH 6.8 and 6.9 respectively for ligamentum nuchae and catgut (cp. Table III). For the action of vertebrate pepsin on these substrates he finds an optimum at

pH 2. Shinoda excludes the presence of pepsin in *Astacus* because of the high optimum which is found here and finds that the juice of *Astacus* can do all that pepsin, trypsin and erepsin can perform together.

Table III. *Proteolytic action of Astacus stomach juice*

O. Shinoda			P. Krüger and E. Graetz		
Method used	Substrate	pH optimum	Method used	Substrate	pH optimum
Alcoholic titration of amino acids	None; autolysis only	3.5, 9.0	Alcoholic titration of amino acids	None	Acid side only
"	Casein	5.6	"	Fibrin	2.7, 6.0-9.0
"	Gelatin	6.5	"	Leucylglycine	7.5-9.0
"	Fibrin	5.5, 8.0	"	Peptone	7.5-8.1
"	Catgut	6.9	"	Clupein	7.0-8.0
"	Glycylglycine and leucylglycine	9.0			
"	Witte peptone	6.7			
"	Peptone F(e carne)	6.2			
Liquefaction of gelatin	Gelatin	5.5, 7.7			
Colorimetric determination of coloured substrate	Fibrin	8.0			
	Ligamentum nuchae	6.8			

Almost simultaneously Krüger (1927 *b*, 1928, 1929) worked on the proteases of *Astacus* and other invertebrates. His results are given in Table III, together with these of Shinoda. As may be seen from this table, there is only a partial agreement between the results of the two authors. Without repeating these experiments it is impossible to decide what is the cause of these discrepancies. The results of the two authors differ especially on the point of autolysis, which is much lower in Krüger's experiments. The minimum of autolysis is, according to Shinoda, situated at pH 6. The condition of the juice may partly cause these differences. Moreover Krüger (1929, p. 557) draws attention to some errors in Shinoda's tables, or at least in the interpretation of his experiments on autolysis.

In this last work Krüger finds the optimum for the "trypsin" at pH 7-8 (on peptone, fibrin, casein, plant casein and clupein). The acid optimum (pH 2.7) of Krüger's former paper (1927 *b*) is not found with improved technique. From the fact that the juice of *Astacus* can attack fibrin, casein and clupein, peptone and dipeptides, Krüger (1929) has concluded that the juice of *Astacus* contains a protease (in the terminology followed in this review, proteinase), a polypeptidase and a dipeptidase. From preliminary experiments with juices and extracts (originating from animals of many classes of invertebrates, he has extended this conclusion to invertebrates in general (1929). In my opinion this conclusion as to the presence of four enzymes is premature. It is based on the supposition that when an unknown

mixture of enzymes splits a certain substrate, which is known to be specific for a certain enzyme, this enzyme must be present. This thesis is right in the case of known enzymes, but is not justifiable if unknown enzymes were present. The various actions which are shown by the unknown mixture may be due to several enzymes as much as to *one* enzyme with quite different properties from all known enzymes. Only separation can give conclusive evidence in this respect.

Attempts in this direction have been made by Wigglesworth and by Yonge. For the proteases of the cockroach, *Periplaneta*, Wigglesworth (1928) found that by means of adsorption the ratio of the tryptic and ereptic actions of an extract of the midgut could be changed. This is a first indication for the existence of at least two definite proteolytic enzymes. Yonge (1930) was able to show the same thing for extracts of the mesenterial filaments of the coral *Lobophyllia*.

Moreover Yonge, in earlier work (1923, 1924, 1925 *a, b*, 1926), described the proteases of many species of different phyla and classes of invertebrates. These proteases were considered mostly in their action on genuine proteins, peptones and dipeptides. For many of them the pH optima were also determined. The investigations show the general occurrence of proteases (as well as of amylases and lipases) through the whole animal kingdom. Yonge has tried to bring the properties of these enzymes into relation with the mode of life and with the structure of the digestive organs of each of these animals. It is difficult to resume the results in a general way, and the reader may consult the original papers (Yonge, 1923, 1924, 1926, 1930, 1937).¹

An elaborate analysis of invertebrate digestive proteases has been carried out for the crab *Maia squinado* by Mansour-Bek (1930, 1932). The quantity of stomach juice per animal being rather small, this author used chiefly a glycerol extract of the midgut gland.

This extract was found to be capable of splitting casein, chloracetyltyrosin, leucylglycylglycine and glycylglycine and consequently the presence of a proteinase, of a carboxypolypeptidase, an aminopolypeptidase and a dipeptidase was suspected. The pH optima for the splitting of different substrates may be seen from Table IV. The autolysis of the extract is very slight over the whole pH range, with perhaps a very doubtful optimum at about 8. The cleavage properties and the optima having been found to be almost the same for juice and extract, the author extends to the juice the conclusions arrived at with the extract. The extract could not be activated by enterokinase, hydrocyanic acid, hydrogen sulphate or zookinase.

After repeated adsorptions with alumina, kaolin and ferric hydroxide at different pH values, it was found that the relative proportion of the actions of the extract on the four different substrates mentioned above varies greatly compared with the original solution. This indicated that these various actions are not due to one single enzyme, but that different enzymes are present. In fact it was possible by prolonged and varied adsorptions to demonstrate the presence of the four enzymes which occur generally in all the complexes of enzymes mentioned above (pp. 252, 254).

¹ *Biological Reviews*.

According to Willstätter (1922, 1926 *a, b*) the adsorption properties of the enzymes depend in a high degree upon the accompanying impurities. Consequently the same procedures would by no means give similar results when applied to different animals. Even extracts from corresponding organs of one species may sometimes need variation in the application of a method (Waldschmidt-Leitz & Harteneck, 1925). It is therefore superfluous to describe here in detail the adsorbents, pH values, etc., which have led to successful results.

Table IV. *pH optima of the proteolytic enzymes of Maia and Murex*

<i>Maia</i>					<i>Murex</i>		
Enzyme	Substrate	Stomach juice	Extract	Pure enzyme	Intestinal juice	Extract	Pure enzyme
Proteinase	Casein	6.1–6.4	6.0	7.4	7.6	8.2	7.9
	Gelatin	6.3	6.1	8.1	—	—	—
	Connective tissue	6.4	—	—	—	—	—
Carboxypolypeptidase	Chloracetyltyrosin	—	—	—	—	7.6	—
Aminopolypeptidase	Leucylglycine	8.2	—	—	—	8.2	8.2
Dipeptidase	Glycylglycine	8.4	8.4	—	—	8.2	—
pH of stomach contents 6.1–6.3					pH of intestinal juice 5.8		

The adsorption behaviour of the enzymes of *Maia* shows one important difference from that of the vertebrate enzymes. In isolating the proteolytic enzymes of vertebrates it has been found by Waldschmidt-Leitz (1929 *c*) that, in the first stages of the adsorption, the tryptic proteinase and the carboxypolypeptidase are similar in their adsorption properties. The same is true for the ereptic enzymes aminopolypeptidase and dipeptidase, and also for the components of the enzyme complex of vertebrate tissue (Waldschmidt-Leitz, 1930 *c*). In *Maia* enzymes, however, the aminopolypeptidase has at the beginning of the separation the same adsorption properties as the proteinase, whereas the carboxypolypeptidase is accompanied by the dipeptidase. This last behaviour of proteinase and aminopolypeptidase has also been observed by Grassmann (1928 *a*) for yeast.

By repeated adsorption the proteinase of the extract of *Maia* has been isolated from all traces of other proteolytic enzymes, whereas the carboxypolypeptidase still retained traces of proteinase, from which it could not be freed because of the small amount of enzyme available. The dipeptidase, like most dipeptidases, was found to be very unstable. Attempts to set the enzyme free from the adsorbates by elution proved unsuccessful. It is doubtful whether the failure of the elution process was due to strong attachment, or to destruction of the enzyme during contact with the adsorbent. The most remarkable feature of the purified proteinase is its activation

by enterokinase and not by any of the other activators mentioned above (hydrocyanic acid, hydrogen sulphide, zookinase). Mansour-Bek concludes from this fact that the proteinase of *Maia* is identical with vertebrate trypsin, or at least very closely related to it. As the extract and the juice are not activated by enterokinase before purification, we must assume that the "trypsin" in *Maia* is secreted, and even present in the secreting gland, in an activated state, whereas in vertebrates the inactive enzyme and its activator are localized in different places. During the purification of the *Maia* enzyme, the activator must have been partially removed from the enzyme by the successive adsorptions, in the same way as this is possible for activated vertebrate trypsin. It is unknown which of these successive adsorptions is most effective in this respect. The purified proteinase is able to cleave pepton (ex. Alb. Merck) and clupein, in which it agrees in specificity with trypsin.

The pH optimum of the purified enzyme has shifted to the alkaline side: from 6.2 to 7.4 for casein, from 6.0 to 8.1 for gelatin. The optimum for vertebrate trypsin on gelatin is situated at pH 8.2–8.7 according to Waldschmidt-Leitz (1924 *b*), and for casein at 8.6 according to Northrop (1923), but at 6–6.5 for casein according to Long & Hull (1917). These statements support the conception of the identity or close relation between vertebrate trypsin and the proteinase of *Maia squinado*.

The stomach juice of *Maia* (like that of *Astacus*) is able to act on connective tissue (ligamentum nuchae). The optimum pH is 6.4. At first this seemed incompatible with the presence of trypsin (as the only proteinase), since it was believed that this enzyme could not hydrolyse this substrate. In vertebrates pepsin alone was assumed to act on connective tissue. Thomas & Seymour-Jones (1923) have found, however, that the supposed resistance of connective tissue to the action of trypsin was a question of pH optimum. The latter is situated for trypsin connective tissue at 5.9, lower than for other substrates. So there can be no objection to ascribing the action of *Maia* (and *Astacus*) stomach juice to the tryptic enzyme.

Krüger (1933, p. 554), in considering Mansour-Bek's results on *Maia* proteinase, is inclined to accept the presence of a kathepsin together with the trypsin-like enzyme. However, the situation of the optimum at the acid side for invertebrate proteinase (Krüger's principal argument), could in *Maia* be changed experimentally into an alkaline optimum by removing impurities. On the other hand Rosén (1935) found a kathepsin in the midgut gland of the snail, *Helix*. It is not impossible that both trypsin-like and kathepsin-like enzymes are present in invertebrates and that the first is secreted and the second intracellular.

Mansour-Bek (1934) has continued the work on the invertebrate proteases by an analysis of these enzymes for the gastropod *Murex anguliferus*. The results for *Murex* agree with those which were obtained with *Maia* in regard to the presence of at least four distinct enzymes for proteolysis. The proteinase, carboxypolypeptidase and aminopolypeptidase each could be entirely freed of other proteolytic enzymes. The dipeptidase is very unstable.

In this paper the kinetics of the proteolytic enzymes were studied more thoroughly, which allowed the establishment of definite enzyme units. The pH

optima of the enzymes of *Maia* and *Murex* can be compared in Table IV, p. 259 and for *Maia* in Figs. 3 and 4.

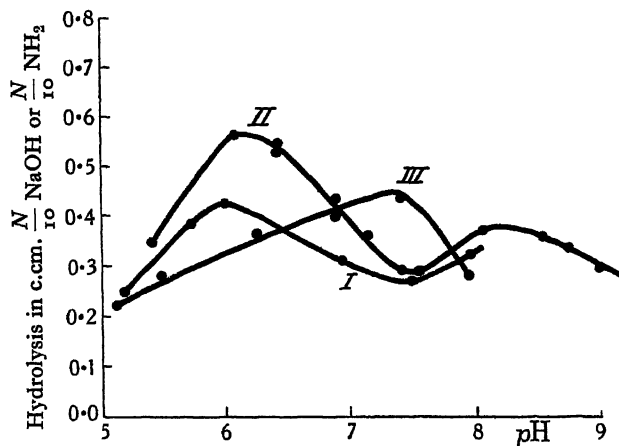


Fig. 3. *Maia squinado*. pH optimum for hydrolysis of casein by unpurified extract of midgut gland (I), stomach juice (II) and purified proteinase (III). The optimum at 8 in curve II (indicated also in I) is due to the presence of peptidases. For the purified proteinase the optimum shifts from 6 to 7.4 and the peptidase optimum has disappeared, the peptidases being removed. After figures of Mansour-Bek (1932), brought to the same scale.

The pH optimum of *Murex* proteinase is shifted slightly to the acid side after purification, whereas in *Maia* it is shifted considerably from the acid to the alkaline side (Figs. 3, 4). In *Maia* the optima of the unpurified enzymes agree fairly well with

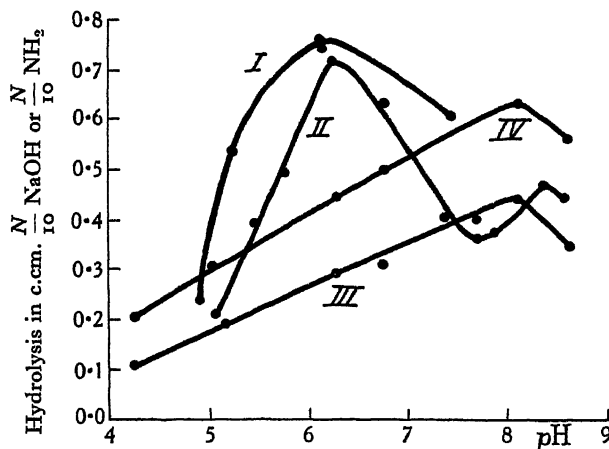


Fig. 4. *Maia squinado*. Same experiments as in Fig. 3, for gelatin. Gelatin + extract midgut gland (I), gelatin + stomach juice (II), gelatin + purified proteinase (III, IV). The same phenomena as in Fig. 3. After figures of Mansour-Bek (1932), brought to the same scale.

the average pH in the stomach (6.1–6.3). This is not the case for *Murex*, as the pH of the intestinal juice (5.8) differs considerably from the optimal activity of the enzymes (see Table IV, p. 259, under *Murex*).

The extracts of *Murex* are not activated by enterokinase or by hydrocyanic acid

nor is the purified proteinase. This does not yet prove that the proteinase is of a different type to that of *Maia*, as it is still possible that the activator here can be separated from the purified enzyme by a special treatment. These experiments have not yet been undertaken.

The distribution of the several enzymes in the different glands of the intestinal tract of *Murex* will be dealt with later.

Hörstadius-Kjellström (see Vonk *et al.*, 1931*b*) has found in experiments not yet published that in the intestinal juice of *Helix pomatia* the weak proteinase must consist of several components: the ratio of the actions on different substrates can be changed by adsorption.

Romijn (1935) investigated the proteases of the cuttlefish, *Sepia officinalis*. Two pairs of glands in the midgut of *Sepia* open into a common duct: the so-called liver and pancreas. Intestinal juice, as well as extracts of these glands, act on the same substrates on which *Maia* and *Murex* proteases showed an action. The pH optima vary from 5.5 to 6, with the exception of those for leucylglycylglycine and glycylglycine, which are situated at 8.3. Adsorption experiments have not been performed. For the action of the pancreatic extract on genuine protein and chloracetyltyrosine, activation by an extract of the coecum is necessary. It could be demonstrated that this last extract can be replaced by purified vertebrate enterokinase, and also that the extract of the coecum can activate inactive vertebrate trypsin. The activator seems therefore to be identical with vertebrate enterokinase, and the proteinase with vertebrate trypsin, which fairly well agrees with Mansour-Bek's results on *Maia squinado*.

Work of Graetz & Autrum (1935) and of Rosén (1932) on invertebrate proteases is only mentioned here for completeness, not having contributed essentially to the problems under discussion.

Rosén (1935) found that the proteinase in the midgut gland of *Helix* is not inhibited by hydrogen sulphide. It can be activated by reduced glutathione, especially if the glands are treated with acetone before extraction, which seems to remove the activator from its combination with the enzyme. This proteinase therefore seems to be of the kathepsin type. The aminopolypeptidase is inhibited by hydrogen sulphide and hydrocyanic acid. The cleavage of peptone by intestinal juice is also inhibited by these agents.

Schlottke (1935) investigated the digestive organs of the king-crab, *Limulus*. He found that the proteinase of stomach juice and of midgut gland could both be activated by vertebrate enterokinase. However, his enterokinase preparation was a simple glycerol extract of vertebrate gut (instead of being prepared by Waldschmidt-Leitz's prescriptions (1924*a*, p. 239)), which must contain a fair quantity of erepsin. This erepsin must be responsible for the observed increase in action, as it continues the cleavage which is begun by the proteinases of gland and juice on casein. That a preparation of this "enterokinase" shows hardly any action on casein is not a sufficient control, for here the erepsin finds no substrate, the cleavage of casein not being introduced by a proteinase. It ought to have been shown that the "enterokinase" showed no action on di- and polypeptides. Schlottke's

conclusion that the proteinase of *Limulus* is closely related to trypsin is therefore quite unproved, although it is probable that the enzyme will turn out upon further investigation to be a trypsin.

Summarizing the results of the last few years for invertebrates, the conclusions which we arrived at for vertebrates, viz. that the protein molecule has to be attacked by at least four different enzymes, are also found to be valid for representatives of two of the big invertebrate phyla. Combining this result with the preliminary results of Wigglesworth and Yonge on other phyla, there can hardly be any doubt that the proteases of all other invertebrates will show the same feature.

As to the *specificity* of the invertebrate proteinase, from the work of Shinoda (1928), Krüger & Graetz (1927-9), Yonge (1923-30), and Vonk (1928), a resemblance to plant and to vertebrate tissue enzymes might be expected. Contrary to these expectations, the work of Mansour-Bek and that of Romijn has shown that the invertebrate proteinase is the same as the vertebrate trypsin, or at least is closely related to it. A kathepsin is also found in the digestive glands, but it is doubtful whether it is also secreted.

The exact nature of the invertebrate polypeptidases and dipeptidases awaits further investigation.

A special problem is the digestion of keratin and other scleroproteins, which are insoluble in water and dilute salt solutions and are not attacked by the known proteinases. However, feeding experiments have made it highly probable that some animals live on scleroproteins. Linderström-Lang (1935) has thrown light on the mode of keratin digestion by the moth, *Tineola biselliella*. Its intestinal contents have a pH of 9.9 and contain a proteinase with an optimum at 9.3. This proteinase does not act on keratin *in vitro*. Now, Goddard & Michaelis (1934) had found that, at pH 10, keratin can be reduced by weak reducing agents and can afterwards be attacked by proteinases. Indeed, after treatment with such agents, the proteinase of *Tineola* attacked keratin *in vitro*. It seemed probable that the proteolysis in the gut had to be initiated by some redox system, which converts the —S—S— of keratin into —SH. (During the digestion the intestinal contents show a marked SH reaction.) By means of redox indicators it could be proved that the redox potential of the intestinal contents does allow the change of —S—S— into —SH groups. The redox system itself, however, does not contain —SH, for by feeding with cotton, imbibed with casein, secretion of enzyme and reducing agent can be evoked with the SH reaction almost absent. It is remarkable that the proteinase is not inhibited by the presence of —SH groups.

(3) *Specificity of carbohydrases*

Among carbohydrases we include the amylases, the cellulases and hemicellulases (considered by some authors to be identical), and the hexosidases. The latter enzymes, substrates of which are better known, will be dealt with first.

The hexosidases are mainly those enzymes which can cleave bioses, trioses (and tetroses). These substrates are generally called hexosides in modern nomenclature; hence the name hexosidases for the sugar-splitting enzymes. For the structure of

sugars the works of Haworth (1929), Pringsheim (1925), Abderhalden (1931, chaps. x, xi), Roger & Binet (1933, p. 436) and of Tollens-Elsner (1935) may be consulted.

The hexosidases are more specific than any other enzymes; Emil Fischer's comparison of an enzyme fitting the substrate like a key fitting a lock is chiefly based on his results with this class of enzymes. Unfortunately the largest part of the literature on sugar-splitting enzymes deals with the enzymes of plants. Adsorption methods have not yet been applied to the disaccharases of animals.

The hexosidases of vertebrates are: saccharase, maltase and lactase. For these enzymes the enzyme-substrate theory, described above for the proteases, is also valuable. Before the substrate is cleaved, the enzyme must attach itself to the substrate. Turning first to saccharase, it is obvious that the enzyme may combine either with the fructose part or with the glucose part of the saccharose molecule. In the first case we speak of a fructo-saccharase, in the second case of a gluco-saccharase.

The saccharase of yeast seems to be a fructo-saccharase (see Waldschmidt-Leitz, 1929 *a*, p. 181). Its action is inhibited by the addition of fructose and not of glucose, whereas animal (vertebrate) saccharase is inhibited only by glucose and not by fructose (Kuhn, 1923 *a, b*, 1925, 1927). This fact we may expect according to the law of mass action if yeast saccharase combines with the fructose and vertebrate saccharase with glucose of the saccharose molecule. Moreover, the saccharase of yeast hydrolyses the trisaccharide raffinose besides saccharose, whereas animal (vertebrate) saccharase can only attack cane sugar (Willstätter & Kuhn, 1921 *b*). This could perhaps be explained by the fact that in raffinose the fructose molecule is situated at the end of the chain, and the glucose molecule is not, so that a fructo-saccharase can combine with raffinose, but a gluco-saccharase cannot. This view, however, is not generally accepted. Euler & Josephson (1924, 1925, 1926 *a*) held the view that the attachment of the enzyme takes place on both sugar residues. The results of Nelson & Anderson (1926) differ from those of Kuhn as to the inhibition by glucose and fructose.

Attempts to purify animal saccharase have not yet been undertaken. Much successful work has been done in this respect on the saccharase of yeast. The optimum for intestinal saccharase is from pH 5 to 7 (Euler & Svanberg, 1921), the optimum for yeast saccharase is situated at pH 4.5.

Saccharase has been found in many invertebrates, although it has never been investigated closely. The pH optimum has been determined by Wiersma & van der Veen (1928) for *Astacus* and found to be 5.7-6.0. It is not known if invertebrate saccharase is a gluco-saccharase or a fructo-saccharase. Bierry (1908-14, see Table V) found that the digestive juice of *Helix* could hydrolyse raffinose as well as cane sugar. This fact might be taken to indicate the presence of a fructo-saccharase. However, it is not at all impossible that a gluco-saccharase is also present there.

The maltase of vertebrates is an α -glucosidase, as will be understood from the structure of this sugar (α -glucosido glucose). This enzyme occurs also in seeds and yeast; in the latter it has been studied more thoroughly. It is also capable of hydrolysing α -methylglucoside and α -phenylglucoside (but not β -glucosides¹). That

¹ A β -glucosidase (or several β -glucosidases) is present in the emulsin of bitter almonds.

these actions are due to one enzyme is concluded from the enzyme-value quotients for the action on maltose and α -methylglucoside and maltose and α -phenylglucoside of different races of yeast. These quotients are identical for the different races. The pH optimum for mammalian maltase has never been determined so far as I know. For the intestinal maltase of the gut of *Testudo graeca*, Wolvekamp (1928) found the pH optimum at 7, Vonk (1927) for *Cyprinus* (pancreas) at pH 6.6-7.1. These optima are rather flat. Maltase has been observed in many invertebrates, nearly always occurring together with amylase. The pH optimum of the *Astacus* maltase is different from that of *Testudo* and *Cyprinus* maltase; it lies at pH 5.3-6 (Wiersma & van der Veen, 1928) and is also very flat. The optima of *Astacus* saccharase and maltase have also been determined independently by Krüger & Graetz (see Krüger, 1929) with the same results.

The lactase of animals is probably a β -galactosidase, since lactose is 1.5 β -galactosido glucose. However, nothing being known of its exact specificity and mode of action, we will abstain from a comparison with the vegetable lactases, especially as few facts are known about them.

In the digestive juices of invertebrates many more hexosidases must be present than in the juices of vertebrates, or at least enzymes must be present with wider specificity than the vertebrate hexosidases. As no attempts at separation have been made, this question is still undecided, but we know, chiefly through the work of Bierry and his collaborators (1908-14), that especially the digestive juice of *Helix* can hydrolyse many more substrates than the hexosidases of vertebrates.

In his work on the cellulase and hemicellulase of *Helix*, Karrer (1925) later on confirmed some of these results, without apparently repeating the experi-

Table V. *Hydrolysis of lower carbohydrates by digestive juices of invertebrates (after Bierry). + pos. - neg. . not tested*

	Dog*	<i>Helix</i>	<i>Aplysia</i>	<i>Homarus</i>	<i>Astacus leptodactylus</i> , <i>A. fluviatilis</i> and <i>Helix aspersa</i>
Saccharose	+	+	+	+	.
Raffinose	-	+	-	-	.
Gentianose	-	+	.	-	+
Gentobiose	.	+	.	.	+
Stachyose	-	+	-	-	+
Lactose	.	+	.	.	.
Lactosazon	.	+	.	.	.
α -methylglucoside	+	+	.	.	.
β -methylglucoside	.	++	.	.	.
Maltose	+	+	.	.	.
Amygdalin	.	+	.	.	.
Melibiosazon	.	+	.	.	.
Melibiose	.	+	.	.	.
Manninotriose	.	+	.	.	.
Rhamninose	.	+	.	.	.
Inulin (polyose)	.	+	.	.	.
α -methylgalactoside	.	+	.	.	.
β -methylgalactoside	.	+	.	.	.

* The experiments with the dog were carried out for pancreatic juice and extracts of the gut.

ments with other substrates. Table V gives a review of some of the substrates which can be hydrolysed by *Helix* digestive juice and the juices of some other invertebrates, according to Bierry's results (1908-14).

We may now turn to the polyases, the enzymes which are able to hydrolyse higher carbohydrates. As such, amylase, cellulase, hemicellulase and inulase occur in animal digestive juices.

What we now call amylase was long known from the action of saliva and of malt extract. The saliva of man, especially, contains a very powerful amylase, accompanied by traces of maltase. In its properties it is almost identical with the amylase of the pancreas. It is only able to cleave starch solutions into maltose, whereas such solutions are totally hydrolysed by sufficiently concentrated acids into glucose. Its *pH* optimum is situated at 6.0-6.8 and is to a small extent influenced by the kind of buffer mixtures used (Ringer, 1912). Animal amylase cannot act without the presence of a small amount of salts (chiefly sodium chloride): amylase which has been dialysed adequately is inactive and can be reactivated by adding 1-3 per thousand sodium chloride (Oppenheimer, 1925-6, p. 694; Michaelis, 1914). In this respect it differs from vegetable amylase, which is active without salts being present. Moreover the *pH* optimum of vegetable amylase lies at a lower *pH* (α , 5.75; β , 4.5; van Klinkenberg, 1932 *a*, *b*). It has been found recently that vegetable amylase can be activated by an amylokinase, whereas this kinase cannot activate animal amylase (Waldschmidt-Leitz, 1931 *c*, 1932 *a*, *b*).

Moreover, recent researches have shown striking differences in the mode of working of animal and vegetable amylases, which have led to a classification of amylases into α - and β -enzymes, as for the hexosidases. These researches began with the work of Kuhn (1924). This author studied the action of malt amylase and of pancreatic and taka amylase¹ on starch solutions. The processes were followed polarimetrically as well as by determining the reducing sugars with a titration method. The polarimetric determinations were made directly after removing the sample from the solution and without removing the mutarotation. Kuhn found that in the case of malt amylase the rotation diminished more rapidly than could be expected from the results with titration (see Fig. 5). After some time, however, the rotation curve reached the same level as the titration curve, if the results of the latter were calculated as rotation of an ordinary maltose solution. In the cases of pancreatic and of taka amylase the results were exactly the reverse (see Fig. 6). The power of rotation diminished here less rapidly than could be expected from the titration values, but it still reached the same level after some time. No other interpretation could be given to these results than that the maltose was primarily set free in the case of the malt amylase as β -maltose and in the case of pancreatic and taka amylase as α -maltose. Afterwards these β - and α -forms must be transformed into the equilibrium mixture.² The parts of the rotation curves on

¹ Taka is a preparation of the fungus *Aspergillus oryzae*, which occurs together with a kind of Japanese yeast.

² The equilibrium mixture of α - and β -maltose contains 36 per cent. of the α - and 64 per cent. of the β -form. The specific rotations are for α -maltose +168°, for β -maltose +118°, and for the equilibrium mixture 136°; for starch about +205°.

the right halves of the figures correspond with the curves of mutarotation for a solution of β - and α -maltose. Kuhn therefore called the amylase of the malt β -amylase and the enzyme of the pancreas and taka α -amylase. It is not meant by these names that the α -amylase hydrolyses α -linkages and the β -diastase β -linkages. Recent investigations of Klinkenberg (1932 *a, b*) have added some interesting facts to these results. Klinkenberg continued the work of Wijsmann, who concluded as early as 1889 that there are two kinds of amylase in malt. The two amylases distinguished by Wijsmann are, according to Klinkenberg, identical with Kuhn's α - and β -amylase. They can be separated by fractioned precipitation with alcohol.

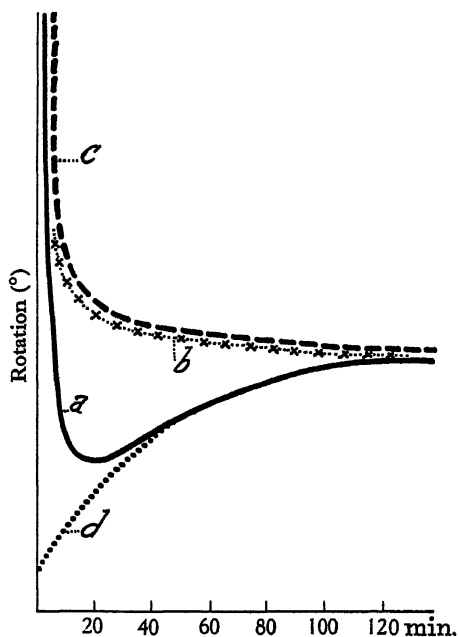


Fig. 5.

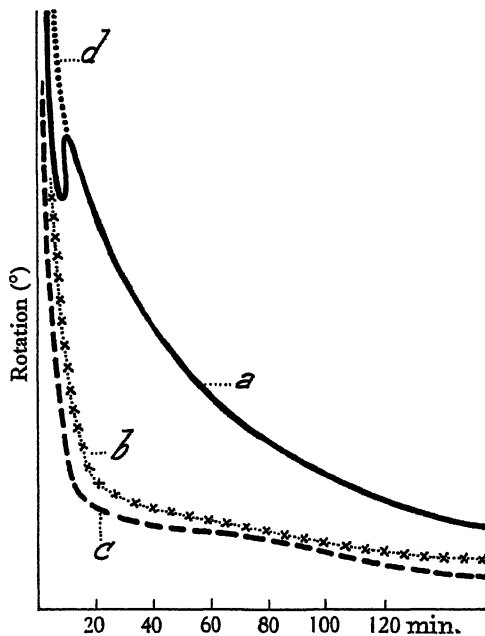


Fig. 6.

Fig. 5. Action of malt amylase on starch, after Kuhn (1924). *a*, direct polarimetric observation; *b*, polarimetric observation after addition of soda to remove mutarotation; *c*, reduction (titrated) calculated in terms of rotation; *d*, mutarotation of β -maltose.

Fig. 6. Action of pancreatic amylase on starch, after Kuhn (1924). Explanation as in Fig. 5; *d*, mutarotation of α -maltose.

That Kuhn finds only a β -amylase in malt is due to the fact that this enzyme is present in the largest quantity. According to Klinkenberg, α -amylase can hydrolyse 36 per cent. of a starch preparation, with disappearance of the iodine reaction; β -amylase is able to hydrolyse 64 per cent. of such a preparation. The iodine reaction does not entirely disappear in this last process. It is peculiar that these values agree with the values of 36 and 64 per cent. for the α - and β -forms present in solutions of glucose and of maltose. This fact leads to the supposition that α - and β -forms, which are hydrolysed by α - and β -amylase, may be present in starch also. After the action of β -amylase a product remains that gives the iodine reaction and has no reducing properties. This product must be the α -form of starch.

It is hydrolysed by α -amylase (though not totally). α -amylase is also capable of hydrolysing glycogen to a large extent; β -amylase not so. Therefore Klinkenberg supposes the α -form of starch to be identical with glycogen. This is in agreement with the fact, which has long been known, that animal amylase, which is according to Kuhn α -amylase, is able to hydrolyse glycogen.

If these facts are confirmed for other animal amylases, they must have some peculiar biological consequences. If animal amylase acts on α -starch alone, animals would be capable of utilizing only the smaller part of the starch contents of their food (in fact, as we have seen, only the α -form). However, some reserve must be made with regard to this conclusion. The animal amylases have not yet been studied carefully in this respect (the amylase of saliva not at all¹). Furthermore Klinkenberg's α -malt amylase can cleave a much higher percentage of starch, if previously activated by heating. Moreover the α -amylase of animals does not seem to be quite identical with that of malt. Waldschmidt-Leitz *et al.* (1931 *c*, 1932 *a, b*) found that α -amylase of malt requires activation by an amylokinase. This kinase is without effect on animal amylase.

In considering the action and specificity of amylases still another point must be borne in mind. The experiments which have been carried out in this respect have nearly all been done with solutions of so-called soluble starch (which is starch previously treated with cold hydrochloric acid to remove the amylopectin of the starch grains). By boiling this product, a clear colloidal solution of starch is obtained. The solution gives the iodine reaction and has hardly any reducing power; the shape of the starch grains, however, totally disappears. It is true also that the carbohydrate food of man is used in a boiled (potatoes) or baked state (as bread, etc.). These processes attack the wall of the starch grains and even produce a certain amount of sugar (*lit.* Shinoda, 1932). The food of animals (with few exceptions among domestic animals) is taken raw. The question is whether their amylase (from saliva or pancreas) can attack starch *grains*. Part of these animals chew their food, some very thoroughly, but others hardly chew at all. Now, it has been found that animal amylase cannot attack intact starch grains. This fact was stated already by Hammarsten (in a Swedish paper) in 1871.² Oppenheimer (1925-6, pp. 667 ff.) mentions that the starches of barley and wheat are digested quite quickly at ordinary temperature by amylase, but potato starch hardly at all. Ehrmann (1913) and Ehrmann & Wolff (1913) found in experiments on dogs that unboiled starch is only slightly digested; 60 per cent. of potato starch is found again in the faeces. Further-

¹ While this article was being written this point has been investigated by Vonk & Braak (1934). They determined the limits of decomposition for the action of amylase of saliva (man) and pancreatic extract (pig) on starch. They found these limits far higher than the 36 per cent. which van Klinkenberg found for α -amylase of malt. Saliva could split up the starch easily to 79 per cent., pancreatic enzyme to 54 per cent. Klinkenberg's results for α -malt amylase, therefore, cannot be extended to animal amylase. The expectation that animal amylase would be able to split up only about one-third of the starch, based on van Klinkenberg's results with malt amylase, has therefore not been confirmed. Roos & Romijn (1934) as well as Giesberger (1934, 1935) came to the same conclusions. The explanation of the differences between the various kinds of amylase awaits further investigation.

² Recently Romijn (1933) and Roos & Romijn (1934) confirmed that raw starch is not digested by the dog, or by pancreatic extracts of dog and pig.

more, the mode of preparation has some influence on its digestibility. Rice flour is digested somewhat better than rice itself. If a diet of meat is given to a dog for a week and afterwards changed to starch, the starch is better digested at the end of the second week than at the beginning. This indicates an adaptation to the changed diet. Probably this adaptation is performed by change in the bacterial flora of the gut. During the second week the flora changed from excess of rods into excess of cocci. Older animals can digest starch better than younger ones. The result of these considerations is that only animals which chew their food well, so that the starch grains are crushed, can digest their starch food. Animals which do not chew their food thoroughly would only be able to digest glycogen with their amylase. The starch in this case could only be digested by bacteria or by the enzymes present in the food.¹ Moreover, it must be borne in mind that in a group of animals which chew exceedingly well, the ruminants, the decomposition of carbohydrates (including cellulose) is chiefly performed by bacteria in parts of the stomach. In accordance with this fact the amylase in their pancreatic extract is weak (*lit.* see Vonk, 1927, p. 491).

Furthermore, it must be emphasized that results with human saliva must not be generalized for other mammals. Actually human saliva is the strongest of all in its amylolytic properties. Besides man, only monkeys, pigs and some rodents have a strong amylase in their saliva. Most of the other mammals, so far as investigated, have hardly any amylase in the secretion of their salivary glands (Bethe, 1927, p. 941).

The question of the digestibility of intact starch grains was taken up by Ullmann (1932) for invertebrates. The investigation was carried out on *Tenebrio*, *Helix*, some caterpillars, *Periplaneta* and *Astacus*. Boiled soluble starch is digested, unboiled soluble starch is not. Ordinary starch crushed with quartz sand is digested; if not crushed, it is not digested. The conclusion of the author therefore is that these animals can digest starch only in so far as the grains are crushed in chewing.

Identity of the invertebrate amylase. This amylase does not differ from vertebrate amylase in its specificity: it hydrolyses starch to maltose. The *pH* optimum varies according to the reaction of the juice. This reaction being mostly faintly acid (*pH* 5.5–6.0) the *pH* optimum is situated generally in this region. For some animals, having an alkaline intestinal juice, the *pH* optimum for the amylase is situated accordingly. For the silkworm Shinoda (1930) found a *pH* optimum at 9.5, whereas the intestinal contents had a *pH* of 9.8. (The juice was collected by making the animals vomit on a Buchner funnel, by narcosis with chloroform.) Activation of invertebrate amylase by salt ions is possible (Lowartz for *Astacus*, 1919). Yonge (1926) investigated the amylase in the crystalline style of *Ostrea edulis* in this respect. He extracted the styles with toluol water, precipitated this (approximately 1 per cent.) extract with absolute alcohol, filtered and washed the filtrate with alcohol. By this procedure most of the salts (especially sodium chloride) are removed and the precipitate (chiefly globulin), which

¹ In fact the enzymes present in the food seem to play a very important role. Therefore the conditions of carbohydrate digestion cannot be judged from experiments with pure starch grains, this method being too artificial. Digestion of starch grains in natural food must probably be initiated by the enzymes of the food.

contained the enzyme in adsorbed form, can be redissolved in water. This solution proves to be nearly inactive with starch solution. It can be reactivated by sea water. These experiments were confirmed by dialysing the style extract. After dialysis the solution is nearly inactive. Addition of sea water causes reactivation. The activation can also be performed by means of different salts. Chlorides (or bromides) are the most effective. The nature of the kation has no influence. Less effective are iodides, nitrates and carbonates. Sulphates and fluorides are ineffective. These results agree fairly well with the observations on vertebrate amylase (see Oppenheimer, I, 1925-26, pp. 694-9). The amylase which can be extracted from the digestive diverticula of the oyster has in this respect the same properties as the amylase of the crystalline style. Krüger & Graetz (1928) have made it probable for *Astacus* that its amylase is an α -amylase. However, their experiments not being very numerous, confirmation and extension of the investigation to other invertebrates is desirable. From a merely speculative point of view it is not improbable that some invertebrates possess a β -amylase. For we have seen that invertebrates, and especially *Helix*, are well provided with enzymes which are able to hydrolyse β -disaccharides (β -hexosidases).

The temperature optimum of *Astacus* amylase is situated at about the same temperature as that of vertebrate amylases. Lowartz (1919) found this optimum for *Astacus* at 45°, Wiersma & van der Veen (1928) at 45-50° C.

Amylase and maltase, which mostly occur together in invertebrates, have not been isolated from these mixtures as two definite enzymes. As, however, Yonge (1926) found amylase without maltase in the crystalline style of *Ostrea*, and thus the existence of an independent amylase for invertebrates is sure, we are certainly not wrong in considering amylase and maltase in their mixtures as two different enzymes.

Concerning inulase, no facts are known other than its presence in the juice of *Helix* and some other invertebrates (see Table V, p. 265), and that it hydrolyses inulin into fructose.

Cellulases and hemicellulases. The faculty of hydrolysing cellulose and hemicelluloses is lacking in the digestive organs of vertebrates. In so far as these compounds are digested there, this is done by the action of bacteria.

Some invertebrates, however, are capable of cleaving these substrates in their digestive tract. The presence of such a cellulase was first discovered by Biedermann & Moritz (1898) in the snail, *Helix pomatia*. Since that time this fact has been confirmed by many observers for different invertebrates. Soon after Biedermann, the researches of Bierry (1908-14) drew renewed attention to this fact. Bierry found such enzymes present in *Helix pomatia*, *H. aspersa*, *Astacus fluviatilis* and *A. leptodactylus*, *Homarus vulgaris* and *Lumbricus*. However, his work was chiefly devoted to discovering the specificity of the enzymes for the lower carbohydrates, as we have already mentioned.

In 1923 Karrer (1923-30) took up the study of the hemicellulose lichenin, and of the ferment in the digestive juice of *Helix* which could hydrolyse it. First he detected that lichenin, prepared from the cell walls of *Cetraria islandica*, could be totally hydrolysed to glucose by means of hydrochloric acid (Karrer, 1923 a).

Contrary to cellulose, it is possible to prepare the lichenin as a powder, which yields a colloidal solution with water. This solution is entirely hydrolysed to glucose by the digestive juice of *Helix* (Karrer, 1923 *b*). In this fact the lichenin differs from other hemicelluloses. The enzyme must probably consist of a hemicellulase and a cellobiase. (From cellulose, which is also entirely hydrolysed to glucose by hydrochloric acid, the disaccharid cellobiose could be obtained as an intermediate product if the cellulose were previously acetylated.) In subsequent work Karrer (1924 *a*) succeeded in purifying the enzyme. By dialysing the juice for 2-3 days, invertase, maltase, lipase, diastase and inulase disappear and the remaining enzymes are lichenase and cellobiase. The procedure is connected with a great loss of lichenase. Finally the enzyme can be freed from further impurities by means of adsorption. After this the enzyme can be precipitated by means of acetone and dried by treating with ether. The pH optimum is situated at 5.28.

If solutions of the enzyme are kept standing for a fortnight, the activity diminishes until a limit is reached, after which it remains constant. This indicates the presence of two enzymes, of which the one which acts on the higher organized substrate remains unaltered, whereas the one cleaving lower products deteriorates (Karrer, 1924 *b*). This view proved to be right in so far as cellobiose, which is hydrolysed before the solution is allowed to stand, is not attacked any more after 14 days. This seemed to indicate that in the decomposition of lichenin to glucose by the juice of *Helix*, cellobiose would be formed as an intermediate product, which in its turn is hydrolysed by cellobiase. If this were true, it would be possible to find cellobiose when an enzyme solution which has been kept standing to remove the cellobiase is allowed to act upon lichenin. This expectation, however, proved to be erroneous. In performing the experiment cellobiose could not be detected, but a new sugar was found, which was called lichotriose. It is a triose and its osazone can be distinguished from all known osazones by the difference in the shape of its crystals and in its melting point (Karrer, 1925 *b*). Either an enzyme capable of hydrolysing this lichotriose must be present in the fresh juice, together with the cellobiase, or the cellobiase itself must be able to do so.

The preparations which hydrolyse lichenin are also able to cleave cellulose, though this is attacked with greater difficulty. Karrer considers this to be due to the insolubility of the cellulose and not to cellulase and hemicellulase being different enzymes. Cellulose and lichenin are considered as chemically different, though closely related substances. As the kinetics of the action on cellulose and hemicellulose are in principle the same, the identity of cellulase and hemicellulase is probable. Attempts to separate the components by means of adsorption have not been made. Probably the quantities of juice were too small for that purpose. For the action on native cellulose, undiluted or even artificially concentrated juice is necessary, so that adsorption experiments would demand large amounts of juice. Karrer continued to speak of cellulase and hemicellulase, although no clear evidence was found for each of them being a separate enzyme. As substrates for the action of true cellulose, the following have been used: cotton powder (precipitated from a solution of cotton in different solvents), filter paper, cotton

wool, and different kinds of artificial silk. Filter paper is hydrolysed more easily than cotton wool freed from fat (Karrer, 1925 *c*, 1926 *b*). For the precipitated celluloses the means of solution and precipitation are of great influence on the digestibility. The commercial products of artificial silk also show considerable differences in their resistance to the enzyme. This does not seem to be due to the chemical properties of the precipitates and products, but more to their colloidal state and fibrous structure (cf. for cellulase Ziese, 1931).

Chitinase. Chitinase, which has been occasionally found in some animals, has been studied more closely by Karrer (1929 *a*, *b*, 1930) in the juice of *Helix pomatia*. Finely powdered chitin of lobster is weakly attacked by the juice. Reprecipitation from cold hydrochloric acid (which causes no change in structure) largely diminished the resistance against the juice. At the beginning of the reaction the Schütz rule is followed. Doubling the quantity of the juice gives an increase in action of 1.3–1.5 times (analogous to the behaviour of cellulase). The cleavage product is *N*-acetylglucosamine (which had also been found previously after the action of hydrochloric acid on chitin).

In heating chitin with concentrated potash, chitosan is formed. This is also attacked by the juice and causes the formation of tri- and tetraglucosamine. In these products the acetyl groups are lacking. After reacetylizing chitosan, the product can be hydrolysed by the juice again into *N*-acetylglucosamine, so that probably chitosan is a high product, which differs from chitin only by loss of acetyl groups. If chitosan is acylated with other acids, such as formic, propionic, butyric and benzoic, the products are no longer attacked by the juice, so that the chitinase seems to be very specific in its action.

It may be mentioned that Oshima (1931) found an enzyme in *Haliotis* which cleaves alginic acid, and one which digests agar. According to the author these enzymes are of significance for animals feeding on brown algae.

We might also expect special adaptations in animals living on wood. This question having been treated for insects by Mansour & Mansour-Bek (1934 *a*),¹ we may refer the reader to this article. Wood consists on the average of about 50 per cent. cellulose, and this component is attacked by the cellulases of wood-feeding animals, if such enzymes are present. Boynton & Miller (1927) showed the presence of a cellulase in the midgut gland of the shipworm, acting on filter paper and sawdust. It is absent in the crystalline style. It has not been proved that the enzyme is secreted. Wood-eating animals also derive nutrition from all the substances present in wood which can be digested by their enzymes (Mansour & Mansour-Bek, 1934 *a*).

(4) *Specificity of lipases*

Lipases are able to hydrolyse fats into fatty acids and glycerol. The specificity in this group of ferments is not very pronounced. As we have seen for carbohydrates and proteins, the hydrolysis of the natural substances is performed by other enzymes than that of the lower products and there are special enzymes for the

¹ *Biological Reviews*.

cleavage of different natural and lower substances. True fats are decomposed into higher fatty acids and glycerol, neither of which is further attacked by digestive enzymes. In this respect the decomposition of fatty bodies presents simpler features.

Closely related to the lipases are the esterases, which can hydrolyse esters of lower fatty acids. However, the difference between lipases and esterases is not an absolute one. Their specificity is only relative. Lipases can also hydrolyse esters, but to a smaller extent, and the reverse is the case for the esterases. Thus pancreatic lipase (formerly called steapsin) is a true lipase, whereas the so-called liver lipase has turned out to be an esterase. Among the esterases there are some which are more or less specific for certain esters, but generally they can hydrolyse some others at a slower rate. Furthermore, we can distinguish between plant lipases and animal lipases.

The first animal lipase to be known was that of the pancreas. It occurs in pancreatic juice and can be extracted from the fresh gland by means of glycerol. As mentioned above, it hydrolyses fats far better than esters. Its *pH* optimum is situated at 7–8 (Oppenheimer, I, 1925–6, p. 471). The enzyme can be separated by means of adsorption from the trypsin and the amylase with which it occurs (Willstätter & Waldschmidt-Leitz, 1923 *a*). Its adsorption behaviour is comparatively non-specific; the enzyme is easily taken up by acid, as well as by basic adsorbents. Its affinity for basic adsorbents is, however, stronger than for acid ones. Contrary to many other enzymes the adsorption properties of lipase do not depend greatly on the state of purity of the enzyme; the adsorption properties therefore remain almost the same throughout the process of purification (Willstätter, 1923 *a*).

Different substances can influence pancreatic lipase as activators. Calcium salts, bile salts and proteins activate in alkaline media, whereas in acid media they are indifferent or even inhibiting (Willstätter, 1923, 1923 *c*, older literature cited there). Magnesium chloride has an extremely strong activating influence in an alkaline medium (Pekelharing, 1912). That the action of lipase is so sensitive to activation can probably be explained by the circumstance that the substrate is insoluble in water, whereas the substrates of other enzymes can give at least a colloidal solution with water. The substances which can activate or inhibit may do so by a change in the adsorption relations. In comparing the action of different lipase solutions or preparations, the presence of such activators must be taken into account.

The study of lipases and esterases has given evidence that the specificity of an enzyme may be influenced by factors that influence the colloidal carrier (Bamann & Laeverenz, 1930 *a, b*, 1934). Kraut (1934 *b*) succeeded in converting pancreas lipase with an excess of active groups (agon) and shortage of carrier (pheron)¹ into liver esterase by mixing it with a preparation of liver esterase with excess of carrier and shortage of active groups. The conversion was proved by the fact that the resulting preparation showed an increase of action on methylbutyrate and a decrease with tributyrine. The reverse experiment also succeeded. Therefore the pheron not only carries the active group, but also plays an important role in influencing its specific action.

¹ Cf. pp. 250, 251.

From the stomach wall a lipase has been extracted, which has its optimum between pH 4 and 5. This enzyme has long been taken for a ferment quite distinct from the pancreas lipase. However, Willstätter & Memmen (1924 *a*) have shown that the different pH optimum is due to accompanying substances. By means of adsorption the stomach lipase can be freed from them and by this purification the pH optimum shifts to the value for the pancreas lipase (8.6). So the enzymes seem to be much more closely related than had previously been suspected. However, their stereochemical specificity is not entirely the same (Willstätter, 1924 *b*).

The same facts have been found by Haurowitz & Petrou (1925) for human stomach lipase. These authors also compared the working and the pH optimum of stomach lipase of many vertebrates, and tabulated the data. In their tables there also occurs a value for the "stomach lipase" of the carp. It must have been unknown to the authors that the carp does not possess a stomach. The bile duct and pancreatic duct in this animal open immediately after the oesophagus and the structure of the proximal wider part of the intestinal tube is that of an intestine and not that of a stomach. The values given in their table for the carp, therefore, are due to pancreatic lipase adsorbed to the intestinal wall (for anatomy see Biedermann, 1911). The optima of this lipase in *Cyprinus* is on the alkaline side, as is the case for the pancreatic lipase of mammals.

There are some peculiar facts in the situation of the optima found by the authors mentioned. Thus, the optimum of the stomach lipase of the pike, *Esox*, is on the alkaline side and also that of all the birds which have been investigated. For most mammals the optimum is situated at the acid side, although for a certain number of them an alkaline optimum is found. The biological significance of these differences is not clear, as the situation of the optimum is not connected with the kind of food eaten by the animal.

Not very much is known about the exact nature of invertebrate lipase. This is not strange because the main points concerning the specificity and the mode of action have only been elucidated since 1934 (Kraut, 1934 *b*) for vertebrate lipases, so that a definite basis of comparison was lacking. The formation of fatty acids from olive oil was stated by Hoppe-Seyler (1877) to occur in *Astacus*. Afterwards several authors used the colour change of milk to which an indicator was added as a test for the presence of lipase. In this way lipase has been found in the digestive juices of many invertebrates.

Krüger & Graetz (1927 *a*) dedicated a separate paper to the lipase of the freshwater crayfish. According to their investigations the enzyme of the crayfish seems to be an esterase rather than a lipase. The esters of lower alcohols and acids are split up much more readily than those of higher ones and true fats.

In a later work Krüger (1929) confirmed the esterase nature of the digestive enzymes of some other invertebrates (*Aplysia depilans*, *Octopus vulgaris*). Yonge (1925 *b*) had observed these facts in *Ciona intestinalis* before Krüger. However, for some species (*Homarus vulgaris*, *Palinurus vulgaris*) Krüger (1929) found a stronger action on fat (oil) than on esters. It seems that in the same species it is possible that sometimes the action on fat, sometimes that on esters, is the strongest.

The causes of these variations are unknown. The biological significance of the esterase nature of the enzyme is not clear. As far as is known, the fats of invertebrates (Fürth, 1903, pp. 232, 568) are different in their composition from those of vertebrates, but these differences are by no means so great that they could justify the presence of a special esterase in the juices of animals with invertebrate food.

Krüger & Graetz (1927 *a*) also found that the quotient for the action of different samples of the juice on methylbutyrate and phenylacetate showed considerable differences, without being able to give a definite explanation of this fact. The *pH* optimum for the cleavage of tributyrin by the juice of *Astacus* is situated between 5.2 and 6.5. This optimal zone nearly corresponds to the zone of *pH* changes in the juice. From the situation of the optimum we cannot draw conclusions as to the identity of the lipase. Experiments with inhibition gave no conclusive evidence on this point. In some ways the *Astacus* lipase showed a similarity to the pancreatic lipase of the vertebrates, in other points it resembled liver esterase and serum lipase.

Kuntara (1934) studied the properties of *Helix* lipase more closely. Unlike vertebrate lipase, it is not much influenced by ions. Its action on different substrates decreases in the following order: tributyrin, methylbutyrate, *dl*-mandelic acid ester, castor oil, olive oil. In this respect the enzyme occupies an intermediate position between lipase and esterase. It also partly agrees with pancreatic lipase in inhibition by different substances, partly with liver esterase. The enzyme could be purified by adsorption and freed from amylase. The *pH* optimum (6.7) is hardly changed by this purification.

The wax moth is able to live on wax. Wax is a mixture of free fatty acids ($C_{24}H_{48}O_2$ — $C_{30}H_{60}O_2$), esters of higher alcohols (chiefly mylissyl palmitate) and higher hydrocarbons. About 40 per cent. of the wax is taken up, the other 60 per cent. appearing in the faeces. The hydrocarbons are not resorbed. Rather more of the free acids is taken up than of the esters (Duspiva, 1935). How can these esters be digested? Pertzoff (1928) observed an increase in acidity when extracts of the moths acted on wax emulsions and ascribed this to the action of a cerase. Kraut (1934 *a*) pointed out that this increase was due to autolysis of proteins in the extracts. With rigorous control experiments no increase is observed. Tributyrin and methylbutyrate are hydrolysed easily. Duspiva (1935) observed the same facts. Myrisin is not cleaved, olive oil and castor oil are cleaved less well than tributyrin and methylbutyrate. It is possible that an activator is present in the gut which is lacking in the extracts. As we saw, such is the case for the digestion of keratin (reduction system).

About the same percentage for the assimilation of wax contents has been found by Dickman (1933). He found no action of larval extracts on wax, but the bacteria of the gut exhibit such action *in vitro*.

(5) *The specificity and distribution of enzymes in general*

In addition to the descriptions given, we must mention the fact that proteases, carbohydrases and lipases of an invertebrate juice have never been separated from each other by means of adsorption, as has been done for the pancreatic extract of vertebrates. The only exception is the isolation of lipase by Kuntara (1934). The adsorption experiments of Mansour-Bek (1932, 1934) were only undertaken for the discrimination of the different *proteolytic* actions of the mixture. We nevertheless consider the actions on proteins, carbohydrates and fats in invertebrates as due to separate enzymes. We do so because cases are known where one or more of these activities is lacking, others being present. A protease is almost lacking in *Helix* and lamellibranchs, whereas an action on many carbohydrates and on fats is present. We find an action on starch alone in the crystalline style of lamellibranchs. The juice of *Helix* shows an action on many more hexosides than that of any vertebrate. Moreover the conception of the existence of different enzymes for all these actions is supported by the fact that in the vertebrates artificial separations of these enzymes have been performed. But it cannot be emphasized enough (as has already been pointed out on p. 247) that our knowledge of the existence of definite specific enzymes has arisen from the natural localization of the digestive enzymes in vertebrates and some other separately occurring enzymes. Here the enzymes are present in a state of natural isolation, which can only be attained in most other cases (plants, invertebrates) by highly artificial adsorption experiments. If we had no knowledge from independently occurring enzymes in their natural state, we could hardly deduce the independent existence of several enzymes from adsorption experiments alone.

The general impression which we get from the facts is that there is much uniformity throughout the animal kingdom in the identity or specificity of the digestive enzymes. Exceptions to this uniformity are pepsin, which is typical for nearly all the vertebrates and for these alone, and the occurrence of cellulose- and hemicellulose-splitting enzymes in some invertebrates. The latter have not been found in any vertebrate. Sometimes they are accompanied by several hexosidases, which never occur in vertebrates, and apart from invertebrates only occur in plants.

Amylase is present in nearly all animals which have been investigated for its presence. Mostly it is accompanied by maltase. Lipases (esterases) are also generally present. Cellulase and hemicellulase are only found in a limited number of invertebrates. Proteases are also widely distributed. But they may be lacking, or nearly so, in some invertebrate juices, e.g. in lamellibranchs and in *Helix* (*lit.* Jordan, 1913) and in holothurians (Oomen, 1926). In the latter, proteases of amoebocytes seem to play a part in the digestion of proteins (Oomen, 1926).

III. LOCALIZATION OF ENZYMES IN THE GUT

In the foregoing treatment of enzymatic specificity we have seen that one of the first and probably the safest means for distinguishing different enzymes is their localization in different parts of the vertebrate alimentary canal. The digestion of proteins is begun by pepsin in an acid medium in the stomach. It is continued by trypsin and erepsin in a neutral medium over the whole length of duodenum and ileum. That of the higher carbohydrates is begun by saliva (man, monkey, pig, rodents) in mouth and stomach (through the situation of the food layers saliva can continue its action there for a certain time). It is continued by the amylase of the pancreas in the duodenum and finished by the disaccharases in duodenum and ileum. This localization of the digestive enzymes in different places is one of the chief points in which the digestion of vertebrates differs from that of invertebrates, as was first pointed out by Krüger (1926) and by Jordan (1927). In most invertebrates (in as far as they possess a well-developed intestinal tract) almost the whole of the digestion takes place in a single part of this tract, which can be a "stomach" (not morphologically homologous with the vertebrate stomach), a gizzard, the midgut, or digestive diverticula. Although different glands may contribute to the production of the digestive enzymes, all these secretions are generally conducted to the same part of the digestive tract and mixed up there. Therefore at the time of the first investigations on the digestion of invertebrates the view was put forward that the digestion of proteins to amino acids could probably be performed by a single enzyme (Jordan, 1907). We have seen, however, that the chemical means of attacking proteins seem to be essentially the same for vertebrates and invertebrates. The difference lies in the fact that in invertebrates all these enzymes occur together, whereas in vertebrates they succeed each other. The localization in the latter, however, is not complete, as tryptic proteinase, protaminase and carboxypolypeptidase are secreted together in the pancreatic juice and aminopolypeptidase and dipeptidase together in the secretions of the gut wall. Moreover, traces of erepsin are present in the pancreatic juice, so that four, or, including the protaminase, five enzymes are present in this juice, though the lower components are in very low concentration.¹

The carbohydrases in vertebrates also succeed each other in a chain along the alimentary canal (amylase with traces of maltase in the salivary glands and saliva,² amylase with traces of maltase in the pancreas and pancreatic juice, maltase, lactase and invertase in the gut and its secretion). In invertebrates all these carbohydrases and even more are present in one juice. This is extremely striking in the case of *Helix*, where all the disaccharases enumerated in Table V occur together with cellulase, amylase, inulase and probably a tri- and tetrahexosidase in one juice. Thus vertebrates possess a "chain" of digestive enzymes (as Jordan, 1927, expressed it), whereas invertebrates have a "mixture".

¹ Attention must be drawn to the fact that in some species or even groups of vertebrates a stomach and a pepsin are lacking, for example in Cyprinoidae. The stomach of *Ornithorhynchus* does not seem to have digestive functions.

² Only man, monkey, pig and some rodents have appreciable amounts of amylase in their saliva.

It must be noted, however, that there are invertebrates where a certain localization exists. Thus in lamellibranchs and some gastropods the amylase is not poured out into the digestive tube, but is contained in the gelatinous crystalline style. An extracellular proteinase is almost lacking in animals which possess such a style. Yonge (1926) found for the oyster that the style extract acted only on starch and glycogen. The substance of the style is chiefly a globulin (*lit.* see Jordan, 1913). Yonge (1925a) demonstrated that the pH of the style (which in its highest development is present in a separate style sac) is always lower than that of the stomach juice which originates from the midgut gland. By means of ciliary movements the style is rotated forwardly into the "stomach". There the emerging part is dissolved by the higher pH reigning there. The result is that the amylase is given off to the food in small doses. The whole mechanism is probably a protection against waste of the enzyme, as the alimentary canal is in open connexion with the surrounding water, no sphincter being present at the proximal end of the digestive tract and the selection of the food being performed by the labial palps.

In cephalopods (Romijn, 1935) the liver contains proteinase and polypeptidases, the pancreas only peptidases and inactive proteinase, whereas the enterokinase necessary for its activation is localized in the wall of the cœcum. The amount of dipeptidase in the juice of many invertebrates is much smaller than that in the midgut gland from which it originated. All these facts indicate a certain localization in invertebrates. For the last-mentioned fact Vonk (1928) has assumed a biological significance (protection of the animal against excess of cleavage products).

In corals Yonge (1930) has found that the extracellular enzymes (in the coelenteron fluid) are proteinases alone. We must assume that the digestion of proteins is completed by intracellular peptidases. If this is true, a certain localization is also present here.

The "enzyme chain" in vertebrates is known from studies on mammals (*lit.* Vonk, 1927), reptiles (Wolvekamp, 1928) and to a certain extent birds, though not much attention has been paid to the distribution of the enzymes in the latter.

In fishes (pike and carp) it has been proved by Vonk (1927) that the localization of proteases¹ is the same as in mammals. For carbohydrases, however, differences have been found. In mammals pancreatic extracts contain, besides large amounts of amylase, small amounts of maltase. Intestinal extracts contain larger amounts of maltase than pancreatic extracts. In fishes (carp) there is (besides a very strong amylase) a rather large amount of maltase in the pancreas, though its amount is small compared to the concentration of the amylase. In the carp the amount of amylase in the pancreas is about 50-100 times as large as that in extracts of the gut (equal weights of pancreas and gut on the same volume of the extract) and the amount of pancreatic maltase is twenty-five times larger than that of the gut, which is a considerable difference from the relations found in mammals (*lit.* Vonk, 1927, pp. 493, 494). But the amount of amylase in the pancreas is about twenty-five times more than that of the maltase. Thus for fishes the enzymatic chain of the

¹ In the treatment of specificity we already saw that this author demonstrated that the proteases of fishes are essentially the same as those of mammals.

proteases is as well-developed as that for mammals, but there is no such chain for carbohydrases.

The occurrence of proteases and carbohydrases in chains in the case of vertebrates and in mixtures for invertebrates has been brought into relation with the more intense metabolism of the vertebrates (especially mammals and birds) and their great sensitiveness to changes in the concentration of amino-acids and glucose in the blood (Vonk, 1927, p. 495). The localizations of the enzymes for higher and lower products (especially the occurrence of maltase and erepsin in nearly the whole length of the gut) protects the animal from inundation of the blood with a large amount of sugar and amino acids after meals. For fishes this protection is reached in the case of carbohydrates by the small amount of maltase present in the pancreas as compared with the amount of amylase. In invertebrates such protection does not seem to be necessary. Hemmingsen (1924 *a, b*) demonstrated in *Astacus* that the sugar value of the blood is by no means as constant as in higher animals, and that the crayfish is not very sensitive to inundation of its blood with glucose. But a protection against inundation with amino acids may exist in invertebrates.

Since fats are hydrolysed directly into glycerol and fatty acids without intermediate products, this process can be accomplished by one single enzyme, so that for lipases (and esterases) no chains of enzymes are possible.

IV. SUMMARY

1. The identity of the digestive enzymes found in vertebrates and invertebrates has been discussed.

For the proteases the conclusion is reached that in the vertebrates as well as in the invertebrates (as far as investigated) the protein molecule is attacked by at least four different enzymes, a proteinase, carboxypolypeptidase, aminopolypeptidase and dipeptidase, which can be separated by adsorption. (Recent researches have, moreover, demonstrated the presence of a separate protaminase in the former trypsin of vertebrates.) The proteinase of *Maia squinado* has the same specificity and properties as the vertebrate trypsin. In the midgut gland of *Helix* a cathepsin is present. Further investigations must verify the distribution of these enzymes for other groups. In the vertebrates the action of these enzymes is preceded by that of pepsin (stomach), working in a strongly acid medium. This pepsin is nowhere found in invertebrates.

The nature and distribution of the proteases seems to be the same for all the classes of vertebrates. It is doubtful whether the enterokinases of the vertebrates are entirely identical.

The amylases of vertebrates are, as far as is known, α -amylases. They need activation by salts (chiefly sodium chloride). The amylases of invertebrates resemble those of vertebrates. An inulase is not found in vertebrates, but is present in some invertebrates. Cellulase and hemicellulase (it is doubtful whether they are identical or not) are also lacking in the vertebrates, but are present in some invertebrates. The properties of these enzymes have been fully treated.

The disaccharases of vertebrates are saccharase, maltase and lactase. Of these, maltase is the most common in the invertebrates, occurring together with amylase (though its separation has not yet been carried out). Some invertebrates, especially *Helix pomatia*, are able to hydrolyse many more sugars (also tri- and tetrasaccharides) than the vertebrates can do. These disaccharases are still awaiting further investigation.

The fat- and ester-splitting digestive enzymes of invertebrates have more the character of esterases than of lipases (or an intermediate character), whereas in the vertebrates the reverse is the case.

2. The differences in localization of the vertebrate and invertebrate digestive enzymes have been discussed.

It has been pointed out that in the vertebrates the digestive enzymes occur in chains and attack the food successively, whereas in invertebrates almost all the enzymes meet in the place where the digestion is performed and attack the food simultaneously. Through this localization the action of the pepsin of the vertebrates becomes possible, for which a medium with strongly acid reaction is required, and moreover the organism is protected against inundation with cleavage products.

Only in fishes (and probably amphibia) among the vertebrates is no pronounced chain of carbohydrases present, but amylase and maltase are chiefly found together in the pancreas.

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GENETICS IN ITS APPLICATION TO PLANT BREEDING

By P. S. HUDSON, PH.D.

(Imperial Bureau of Plant Genetics, Cambridge)

(Received 27 March 1936)

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I. INTRODUCTION

THE turning of the last century witnessed an event that was destined to play a role in the study of biology greater than almost any other single event in the whole history of the science—the rediscovery of Mendel's laws. The significance of these laws was appreciated simultaneously by three great biologists, Correns, Tschermak and de Vries, and not more than a year or two elapsed before their equal significance for the science of practical breeding was grasped by Nilsson-Ehle, Biffen and others. The task of the early workers in this field was to convince themselves, and later others, that the laws evolved by Mendel for the inheritance of clear morphological characters were equally valid for more recondite characters such as the economic properties occupying plant breeders tend to be. This validity was demonstrated by Biffen for two of the most important economic characters in English cereals, rust resistance and baking quality in wheat, and the history of plant breeding since that time has been a sequence of attempts, more or less successful according to circumstances, to apply these laws to a succession of different characters in a variety of different plants. The most interesting of these cases, beyond the ones already mentioned, is perhaps the production of Marquis wheat in Canada by crossing the popularly grown variety Red Fife with a very early maturing Indian variety, Hard Red Calcutta. The new variety, Marquis, selected from the progeny was about 1 week earlier in maturity than Red Fife, which it equalled or surpassed in quality

and yield. Marquis entirely superseded Red Fife in the areas where this had previously been grown, and, on account of its earlier maturity, was capable of cultivation considerably farther north. Large tracts of country in the north of Canada were thus brought under wheat cultivation, and over thousands of acres Marquis was the only variety grown for a number of years. By a continuation of the same line of work a succession of new hybrids of ever-increasing earliness has been introduced in later years, Ruby, Prelude, Garnet, etc., which in many localities are in turn replacing Marquis.

The combination by hybridization of malting quality, productivity, early maturity and strength of straw in barley can be illustrated by the production of the two varieties Spratt-Archer and Plumage-Archer. Between them these two varieties now account for more than 75 per cent. of the barley grown in the British Isles, and have done more than any others to establish barley growing on a sound footing in these islands.

The production by similar methods, complicated by the more difficult nature of the plant as a subject for hybridization, of high-quality potatoes immune to wart disease (*Synchytrium endobioticum*), which have now entirely ousted the old susceptible varieties, might serve as a further example, and such instances could now be quoted almost *ad infinitum*.

II. SINGLE PLANT SELECTION

The applicability of the Mendelian laws to the inheritance of economic characters has therefore been amply confirmed: so much so that mere reports of the Mendelian inheritance of a particular character have almost ceased to have any interest. The proof of the hereditary nature of these characters, however, together with Johannsen's theory of the pure line, lies at the root of the principle of selection, which itself is the foundation of all successful breeding. This principle rests on the fact that by repeated self-fertilization and selection a population should be obtained in which all individuals are of identical genetical constitution and are all homozygous, so that further selection has no effect whatever. The aim of all breeding is to establish such a line which shall be pure for all the characters desired (except where special circumstances, such as the possibility of vegetative propagation, make purity unnecessary). It is not without interest to note the number of plant-breeding achievements that have been made by the application of this principle alone, namely, by individual plant selection without recourse to hybridization. In sugar beet, for instance, the result of about 100 years' selection has been to raise the sugar content from 9 per cent. to more than double that figure. Intensive selection of flax, which was carried out in Ireland from the stimulus given by the shortage of seed supplies from Russia, resulted in the production of several very superior strains. These were produced by straightforward selection of what were styled "particularly good plants", and include some very successful varieties such as the famous Stormont Gossamer and Stormont Cirrus. Some of the most valuable results of selection have been in the isolation of disease-resistant lines. This has been achieved in flax

in America by Bolley (1909), who has produced strains resistant to wilt (*Fusarium lini*). The valuable Danish varieties of swedes resistant to club root (*Plasmiodiophora brassicae*) were obtained by selection, and Findlay has produced a resistant turnip in Scotland by the same methods. Similarly, in cotton, valuable wilt-resistant forms of Egyptian cotton have been produced by selection by Fahmy (1931), and the production of the variety U 4 resistant to the jassid insect (*Chlorita fascialis*) has, it is hardly an exaggeration to say, been the salvation of the cotton industry in whole tracts of the African colonies where ravages of the insect had made cotton cultivation wholly unprofitable and, in many places, impossible. The variety U 4 was produced by repeated selection from the progeny of a plant that had attracted attention because of its healthy appearance when all others were suffering from heavy infestation. The new strain proved to be not only highly resistant to the jassid but also possessed many other desirable features, and its cultivation has now extended far beyond the areas for which it was originally designed.

Even cross-fertilized plants are often capable of improvement on these lines; indeed in the United States of America vastly improved forms of maize have been produced by continuous inbreeding, leading to the production of more or less pure lines, which must then be crossed among themselves to restore their vigour. It has even been possible by inbreeding and selection in sugar beet to select lines possessing a high degree of self-fertility, from which it is hoped to select fully self-fertile lines which can thus be maintained in a pure state by inbreeding without the attendant loss of vigour and other difficulties associated with cross-fertilized plants. According to Grinko (1930) and Zossimovitch (1935), self-sterility in sugar beet is conditioned by a recessive gene, and self-fertile lines have therefore been isolated by selecting lines containing the corresponding dominant. This line of investigation should also open up promising possibilities for other crops.

It is evident from these few brief examples that, given suitable material, much can be accomplished by the aid of individual plant selection alone. One of the most striking successes of the method is the production of the sweet lupin. It is well known that the wild forms corresponding to many of the cultivated leguminous plants such as the pea, lentil, vetch, etc., contain an alkaloid in the seed, which is thus rendered unpalatable and even poisonous. The cultivated forms must presumably have originated in the first place from some chance forms without the alkaloid which have arisen among the common wild population, and the possibility naturally suggested itself that, if a rigorous search were made, analogous forms might be found in the lupin, in which so far no "sweet" forms were known. Such a search was made by Sengbusch (1931) in Germany, who succeeded in isolating lines with as little as 0.01 per cent. total alkaloid. The full details of the procedure were, however, not published and the new strains were carefully guarded; they have indeed to this day not been allowed to pass out of Germany. The Russian botanists, therefore, at the Institute of Plant Industry, Leningrad, undertook a search on the same lines and elaborated their own method, which was published in full (Ivanov, 1932), for the rapid estimation of minute quantities of alkaloid in a seed. By means of this method as many as 1000 plants could be tested by one

worker per day, thus making it possible for very extensive observations to be made on all the more valuable species of lupin for agronomic purposes, gathered from every possible source in any part of the world where lupins are grown. Enormous numbers of each species were tested, and the results showed that the alkaloid content of lupins is subject to wide variation, and that the forms of certain regions are characterized by higher alkaloid content than others. Thus in *Lupinus luteus* the alkaloid was low in seeds obtained from Holland and Bremen, these samples containing high proportions of alkaloid-free seeds, some as much as 100 per cent. The samples from most other regions contained few, or, more usually, no alkaloid-free forms. As an illustration of the rarity of the desired forms in cases such as this, it may be pointed out that in *L. angustifolius* only 1 out of 31,591 plants examined, from the most varied sources, was free from alkaloid. In some of the other species examined no form entirely free from alkaloid was found though, in all species, forms with very low alkaloid content appeared. On going into the question of the geographical distribution of these low alkaloid forms, it was found that they tend to be concentrated in the western Mediterranean countries, and a more thorough investigation still of the forms, both cultivated and wild, occurring in these countries, was then undertaken, in consequence of which further sweet forms were discovered. These sweet lupins have been multiplied and have all been found to breed true for the absence of alkaloid. They constitute an exceedingly valuable new source of protein for use both green and as seed for feeding to stock, and the unripe seeds form a new green vegetable for human consumption.

III. GEOGRAPHICAL DISTRIBUTION AND COLLECTION OF BREEDING MATERIAL

The history of the sweet lupin affords an excellent illustration of the new method of plant breeding evolved by the Soviet school. The method is founded on a simple principle which has nevertheless been overlooked by a great many breeders in other countries, namely, that success in producing a given type of plant by breeding must depend on the possession of a suitable choice of material to work with: the greater the range of initial material the greater will be the chances of producing the desired type. The recognition of this fundamental principle has led the plant breeders of the Soviet Union, headed by N. I. Vavilov, to make collections of all the plants grown or likely to be grown in the Soviet Union. These collections are made on the most comprehensive scale by correspondence, exchange and actual expeditions to the countries where the respective plants are grown, and represent the most complete assortment of the plants concerned that have ever yet been made. The plants are not merely assembled in this way, but are classified and subjected to a thorough botanical, genetical and cytological study, so that the collections represent museums of living material, the characters of which are known and available at any time for use as initial material in breeding. Thus 300 different species are now under investigation on these lines, and in wheat alone over 5000 specimens are sown annually, from which the forms best representing the ecological

type of each country are selected as standards and grown in thirty separate breeding stations for more detailed study and utilization. The breeder in the Soviet Union, when confronted with a new problem, avails himself of these collections and can rest assured that if the characters which he seeks exist they are present in the material at his disposal, and that if the problem is capable of solution he is therefore in a position to solve it.

The study of cultivated plants on these lines has shed a great deal of light on the question of plant distribution, and a number of new facts having a very direct bearing on plant breeding have been disclosed. For instance, it has been found that cultivated plants, contrary to previous tradition, are subject to very much the same laws of distribution as wild plants, and that this distribution has in a great number of cases been very little altered by man's agency. Each plant studied has proved to be subject to a quite regular law of distribution, which takes the form of radiation from a given centre. This centre, which usually embraces a relatively limited area of country, is characterized by an exceptional abundance of varieties and forms. Quite often no two forms are alike, and the number to be found in this small confined space far exceeds the total number occurring in the whole of the rest of the area of distribution of the plant in question. The same applies to the number of plant characters present, for a greater number and variety of characters are to be found in this small zone than in all the rest of the area. Many of the characters found in this centre are endemic, being unknown outside the small territory concerned, so that their very existence was unsuspected before this locality was studied. Thus in the case of hard wheats, *Triticum durum*, the area of greatest diversity occurs in Abyssinia, between the altitudes 2400 and 2800 metres. The Soviet expedition to that country in 1926 and 1927 found such a profusion of forms of *T. durum* and *T. turgidum* that existing views on the relationships, systematics and origin of this group of wheats were entirely changed. Many new characters not known to exist in the group were found, such as purple seedlings, the presence of four to six vascular bundles in the coleoptile, smooth awns, keelless glumes, lax ears, awnless ears, free-threshing grain, and many others. The range of variation proved to be greater even than that occurring in the 42-chromosome wheat group: the grain colour varied from purple to white, all possible degrees were found in density, size of grain, ear and glume, in awn length and length of tooth of the empty glumes, in time of maturity and disease resistance; there were also two distinct types of pubescence (hitherto only associated with the *vulgare* wheats), hairy and hairless auricles, waxy and waxless leaves, and many other new characters showed similar unexpected diversity. Many of the characters hitherto associated with *T. turgidum*, *T. vulgare*, *T. persicum*, *T. dicoccum*, and even rye, were found in *T. durum*, and *durum* characters appeared in *T. turgidum*. In other words, the characters usually associated with a number of distinct species appear here together in a population that in many respects resembles a sort of melting pot of the species as we know them, suggesting a primitive population from which the distinct species have not yet separated out.

The characters and genes associated with the centres of distribution are also

found to be primitive in type. They are the ones usually associated with the wild forms or related species of the plant in question, and are genetically dominant to those common in the highly bred cultivated forms. On passing away from the centre of diversity in all directions, there is a diminution in the number and variety of the forms and characters present, and at the same time in the relative proportion of the dominant genes. This diminution continues progressively as the distance from the centre increases, so that at the periphery of the area of distribution we find the opposite features, the occurrence of a very uniform material with little varietal diversity and possessed of almost exclusively recessive characters. Thus at the margin there occur usually not more than one or two forms. However, many of the recessive characters found in the marginal areas are economically of great value, and it sometimes happens that secondary centres develop in these zones, characterized by an almost equally great diversity of recessive characters and forms. Many of these recessive characters are agronomically of great value, such as freedom from alkaloid in the legumes, tough rachis in the cereals, freedom from pigment, and many other features characteristically associated with cultivated plants. Many characters are, on the other hand, highly prejudicial, notably an excessive tenderness and susceptibility to all sorts of diseases and pests, which commonly distinguishes cultivated from wild forms. If a desired character happens to be dominant, no amount of breeding will produce it from the recessive forms, and the real value of the distribution studies is that they have disclosed the existence of these primary centres containing the dominant genes, many of which had not previously been known even to exist, corresponding to the recessive forms with which we are more familiar. It is to these centres that breeders will look when in search of new characters to introduce into their crops. The *durum* wheats may again be quoted in illustration. In Russia, for many years, attempts have been made to produce an awnless *Triticum durum*. The only satisfactory awnless parents to use in crossing were bread wheats, *T. vulgare*, with a different chromosome number, in consequence of which the hybrids exhibited varying degrees of sterility, and the production of the desired type was a matter of great difficulty. With the discovery of the Abyssinian awnless forms the problem became one of simple hybridization and could serve as a schoolboy exercise in simple Mendelism. And so with a number of characters.

The combination of characters normally associated with separate and distinct species is a further characteristic of the forms occurring in the primary centres, and in the case of the 28-chromosome wheats already cited this, combined with other arguments, has led to the conclusion that these forms constitute a relic of some early type existing before the differentiation of the species in the group and which has in fact given rise to the separate species by gradual loss of genes and increase of specialization and differentiation. The course of this evolution can be retraced by following the sequence of forms and characters on passing from the primary centre to the periphery of the area of distribution. It is then seen very clearly that evolution has consisted in the successive loss of genes, whereby an original population containing all the possible genes, and thus preponderantly dominant, has gradually become converted into one containing fewer and fewer,

to the necessary light conditions, etc., for just such a time as to ensure completion of the second stage, the treatments being applied immediately after the seed has begun to germinate. Every variety has its own particular requirements in each developmental stage, which must be determined experimentally before successful treatment can be applied.

✓X. BREEDING EARLY-MATURING FORMS

The advantages of vernalization are not confined to the speeding up of plant breeding by treatment of the young plants. It also has a direct genetical application. Under normal conditions of growth in the field, varieties may be retarded in reproduction on account of unsuitable conditions for the passage of either the first or the second developmental stage. Thus according to Lysenko (1934) one variety may be late in maturity in a given region because it takes too long to accomplish the first stage, while all other stages are capable of passing quite rapidly. Another variety may be delayed in the second stage while all other stages of its development, including the first, proceed rapidly in the district in question. Both these varieties, though for different reasons, may be equally late in maturity. Lysenko claims that by taking two varieties known to be of these respective types and crossing one with the other it should be possible to obtain a form which is delayed in neither the first nor the second stage and so is capable of developing successfully in the given region. He has made over thirty crosses of this nature and in nearly every case has obtained segregates that are earlier than either parental form.

Great emphasis is placed by the new school of Soviet botanists on the necessity for viewing the vegetative period not as a whole but as a series of phases each capable of independent variation and independent genetical treatment. A new line of approach has thus been given to the problem of breeding early-maturing forms and justification of the new approach has been found in an examination of the pedigrees of the most noted early wheat varieties, Prelude, Garnet and Ruby in America and Novinka in Russia. All these reveal in their parentage the presence of one variety from the extreme north, such as Ladoga or Onega, and one southern variety from India or other warm country. The northern parent has a high light requirement, being adapted to the long almost continuous days of the summer in the far north, but has a low heat requirement, being capable of ripening at quite low temperatures of 12°C. or below. The southern variety on the other hand, although having a high temperature requirement, is almost neutral to light. Crosses of these extreme types complementary in a number of physiological characters of this kind usually transgress the parental limits, giving segregates exceeding both parents in earliness and are considered by the Russians as one of the most promising sources of ultra-early varieties, the production of which is a decisive factor in bringing whole new tracts of country into cultivation. The above example offers one more illustration of how the interest in modern genetics is passing from the study of simple characters giving clear-cut Mendelian segregations to the investigation of the more fundamental and infinitely more complicated characters which go to make the success or failure of a variety in agricultural practice.

XI. YIELD

The most important of all these characters is yielding capacity or productivity, which proves to be the most complex character yet studied. 'Breeding for high yield has usually been done empirically by selecting out those lines which give the highest crop when grown in comparative field tests. Very elaborate and accurate methods of doing this have been worked out in recent years mostly by the agricultural statisticians in this country, and the conducting of these tests, together with the statistical treatment of the results, has come to be one of the most exact sciences in the realm of biology. These methods have been reviewed in recent monographs by Wishart & Sanders (1935) and by Fisher (1935). The problem of yield itself, however, has remained obscure, its very complexity having deterred most geneticists from attempting a detailed analysis of it. Engledow and his co-workers (1923) have made elaborate analyses of the individual components of cereal yield. Studies of a similar kind, rather more directly from the genetical point of view, have been made by Heuser (1931) in Germany, and the matter has been the subject of genetical researches in Sweden, where Rasmusson (1933) has calculated that at least 100-200 separate genes are concerned in the inheritance of quantitative characters such as yield, quality, etc.

✓ Rasmusson finds that the effect of a given gene upon a given character is not a constant value but varies with the number of other genes in operation which affect the same character. The effect of any single gene diminishes in proportion as the number of other genes operating increases. This phenomenon is referred to as "interference" and is used to explain a number of peculiar cases of genetical behaviour not capable of interpretation on the basis of plain Mendelism, for instance the failure to produce homozygous forms superior to the parents in crosses between highly improved parents and the unusually large proportion of inferior forms in the progeny of crosses between good but unrelated varieties; or again the delay of one or more generations in the falling off in yield on inbreeding individuals exhibiting heterosis. All these examples are explained on the assumption that "the effect of each factor on the genotype is dependent upon all the other factors present, the visible effect of a certain factor being smaller the greater the number of factors acting in the same direction". Curves are calculated expressing the segregation of different populations for varying degrees of interference. The degree of asymmetry of these curves increases with the interference and the curves are found to agree with the results of a number of actual crosses, including even Nilsson-Ehle's classical case of the three colour factors in wheat grain.

Some of the most careful of all the researches on the genetics of yield have issued from the plant physiology department of the Landbouwhoogeschool at Wageningen in Holland, under Boonstra (1931, 1934). This laboratory represents one of the few places where genetical problems are considered from the physiological point of view, and as yield is obviously a physiological problem a great future may be foreseen for this line of attack. Yield is ultimately determined by

all the physiological processes occurring during the course of development of the plant, these in turn being governed by the genetical constitution of the plant in respect of all these separate processes. Only in that sense is it possible to speak of yield as being genetically determined. Boonstra emphasizes the necessity for considering the fundamental vital processes such as absorption, assimilation, transpiration, translocation of organic and inorganic materials in their relation to yield. In his own experiments, differences of up to 25 per cent. have been observed in the length of life of the leaves in different varieties of oats. This has a very direct bearing on the total amount of carbon assimilation during the life of the plant and so on the total yield. Special attention has been given by Boonstra to the efficiency of the root system. A "root value" is calculated by taking the ratio of the dry weight of the parts above ground to the dry weight of the roots, on the argument that the greater the efficiency of the root system the greater will be the weight of green matter that a unit of root system is capable of supporting. These root values give a single collective index of the result of all the complex physiological processes which in combination constitute plant yield. The root values were calculated for seven varieties of peas, grown under identical conditions. Differences of over 100 per cent. in root value were observed, showing that the root values do represent a clear varietal character. Comparisons of two varieties, one with a high root value and one with a low value, showed that the variety Fr. with the high value absorbed more water and more salts per gram of root than the variety V 38 with a lower value, and the same was true for other varieties. However, for each gram of water absorbed a greater amount of ash constituents was taken up by the varieties with the lower root value, and this difference became more marked in proportion as the soil moisture content became less. The ratio between water and ash absorption was not directly correlated with the root values, which shows that the absorbing surface of the root is not the only factor concerned. The absorbing capacity of the cells for water and for salts must evidently be different. One suggestion for the reason of this difference is a difference in the osmotic pressure and permeability of the cells. It was actually found that the varieties with the low root value absorption had a lower osmotic pressure, and this may possibly furnish an explanation for their greater salt and lesser water absorption.

Somewhat similar studies are now being pursued by Orlovsky & Umanska (1934) in the Soviet Union on sugar-beet and other economic plants. Examinations of the course of development of a number of different types of beet throughout the life cycle have shown that the high yielding beets of the forage type exceed all others in number of leaves and size of leaves (thus in total assimilating surface) during the early growth phases but gradually give place to the sugar beet types towards the period of maturity. Thus at the end of the vegetative period the beets of maximum sugar content are found with a large number of fresh leaves vigorously assimilating, at a time when most of the mangold leaves are already withered or are turning yellow. In other words the maximum leaf activity of the high yielding type falls at a time when vegetative activity is at its highest and that of the sugar type at a time when sugar formation is in the ascendant. Observations of this kind

give the first reliable indications for a systematic planned synthesis of the desired type to replace the empirical methods hitherto in use.

The conclusion which Boonstra draws from the results of his experiments are, firstly, that it is comparatively useless from the breeding point of view to choose parents merely on the score of their final performance as regards yield. The final yield is an end point and gives no indication of how the product is built up. Even such characters as tillering, number of grains per ear, etc., are not truly yield constituents but merely finer subdivisions of this end point. The yield is determined by the combination of all the physiological processes occurring during the life of the plant in all its separate organs and any given yield may be built up in a great number of different ways. For instance, one variety may give a high yield mainly on account of a high assimilation per square centimetre of leaf surface, another because of a long duration of the functional capacity of the leaves, another again as the result of a high absorption capacity for nutrient materials, and still another by dint of rapid translocation of the products of assimilation, and so on. Each of these varieties may be identical or similar in yield and the aim of the physiological breeder should be to analyse the yield in all his varieties and discover the factors that are responsible for yield in each type, making his crosses in such a way as to combine all these contributory yield factors if possible into one individual. Systematic synthesis of this kind is only possible after thorough analysis, as in any other branch of science, and much painstaking labour lies before the breeders of the future before such a paragon will be achieved, but at least one more line of advance has been pointed out whereby the proverbial task of the plant breeder can be accomplished, namely to make two blades of grass grow where one grew before.

XII. SUMMARY

1. The importance of Mendelism in relation to practical breeding problems is illustrated by reference to early work on the inheritance of economic characters.

2. Examples are given of improved varieties that have been produced by individual plant selection. The possibilities of success are shown to be dictated by the completeness of the material and one of the essentials for successful breeding is shown to be the collection of adequate and representative stocks of initial breeding material. This is illustrated largely by reference to Russian work, which has also elucidated the laws governing the distribution of plant varieties, characters and genes.

3. The possibilities of putting interspecific or distant crosses to practical use are analysed and cases where this has been done are cited. Chromosome duplication is shown to be a frequent phenomenon in such cases and to be a factor greatly increasing the economic value of such distant crosses. Numerous cases are quoted and the phenomenon is shown to have played an important role in the origin of new species and forms in nature. Its experimental control is thus of great practical value.

4. The origin of new forms of pathogenic fungi by hybridization and mutation is illustrated and shown to be a serious obstacle in breeding for disease resistance.
5. Examples are given where the obstacle has been overcome by breeding methods and other cases are mentioned where the problem of disease resistance remains unsolved.
6. The role of mutations in the origin of new forms of agricultural plants is discussed and the possibilities of effecting this process artificially are analysed.
7. The phenomenon of vernalization is described, with special reference to breeding. The significance of this and other recent physiological studies in producing forms possessed of earlier maturity is discussed.
8. The nature of plant yield is discussed and reference is made to attempts to analyse and regulate this by genetical methods.

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FISH MIGRATIONS

By E. S. RUSSELL, O.B.E., D.Sc., F.L.S.

(Fisheries Laboratory, Lowestoft)

(Received 4 May 1936)

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I. INTRODUCTION

THE migratory movements of fish are of such great practical importance to the fishing industry that they have been studied with some thoroughness and on an extensive scale; the general movements of some species, for example, plaice and cod, are well established, at least in broad outline. The most satisfactory and direct method for the study of migrations is of course to mark the fish by means of tags or vulcanite buttons, or in some cases by cutting off a fin. (For a review of methods of marking "round" fish see Graham, 1929.) Hundreds of thousands of fish have been marked by the maritime nations of Europe and by the United States of America, and in some species, e.g. the plaice in the North Sea, the percentage recaptured, through the agency of fishermen, is very high—up to 50 per cent within a year. The direct knowledge of migrations built up as a result of marking experiments is supplemented and completed by other methods of investigation, for example, by a study of the times and places of spawning, and by tracing out the drift of the eggs and larvae.

Not all fish can be successfully marked, and where marking is impracticable, migrations can be traced only by indirect methods, as by the study of detailed fishery statistics, which show where and when the fish are most abundant, and sometimes enable us to distinguish the movements of the large mature fish from those of the immature. Age determination of representative samples obtained from fishing vessels and from special hauls by research vessels also helps to throw light upon the broad movements of the fish. The story of the cod and haddock in the North Sea has been worked out largely by these indirect methods, supplemented by marking experiments on a limited scale (see Graham (1934) and H. Thompson

(1922-9)). The same is true of the hake, which is a fish of the Atlantic seaboard, living for the most part in comparatively deep water and for this reason very difficult to mark successfully (Hickling, 1927-35). The herring is another fish which does not take kindly to marking (see, however, Rounsefell & Dahlgren, 1933, and Dahlgren, 1936), and our knowledge of its movements is very incomplete; the various geographical groups are strictly seasonal in their shoaling, and between the seasonal fisheries to which these shoals give rise their movements are extremely difficult to trace.

Speaking very generally, and disregarding known exceptions, the general schema of the migrations of fish is the following. (1) A migration of mature or maturing individuals to a more or less definite area for spawning; this spawning migration is often, but not always, upstream or up-current. (2) A dispersion of the spent fish after spawning, generally downstream or down-current, followed by an eager search for food, which may lead to definite feeding migrations. Forming an essential link in the general cycle, there is also the dispersal of the eggs and larvae from the spawning ground, which is usually, to begin with, a passive movement in the direction of the prevailing current (see Graham, *et al.* 1926) but soon changes over into a more or less active seeking out of the normal habitat by the young fish.

In addition to spawning and feeding migrations there are other mass movements of lesser amplitude, carried out under the influence of seasonal changes, especially of temperature. These are often at the same feeding migrations, as when soles move into the Suffolk estuaries in summer; their movement out in late autumn is probably a response to the lowering of the temperature. A marked offshore migration of soles in the North Sea in response to abnormally low temperatures took place in the early months of 1929, which were unusually cold (Lumby & Atkinson, 1929).

The main thing is, however, the big cycle of movement—spawning migration, generally contranant, and dispersal of spent fish and of larvae, generally denant. The general effect of this is of course to keep the species within its normal geographical bounds, within its ecological norm.

In this article I propose, first of all, to illustrate these broad features of migration by reference to the cases where our knowledge is reasonably complete, selecting for this purpose the cod, the plaice and the salmon. I shall then deal with some other cases of special interest which have come to light in recent years. This article makes no pretence to being exhaustive, nor does it attempt to cover the ground already traversed by Meek (1916) and Scheuring (1929, 1930). It is to be regarded as supplementary to these valuable résumés of knowledge.

II. THE COD

The life cycle of the *cod in the North Sea* can now be described with some degree of accuracy (Graham, 1934, sect. 5). Large numbers of mature cod collect in February and March every year in certain definite areas, the fishing grounds known as the Flamborough, the Forties, the Great Fisher Bank and the Ling Bank, lying to the north and west of the Dogger Bank. There is a concentration also in the

Moray Firth. Spawning takes place in February to April, but mainly in March, several millions of eggs being liberated by each female. After spawning, the large cod scatter in all directions and resume active feeding. From about June to September they pursue the herring, and their distribution follows closely that of the herring along the western side of the North Sea, as far south as Flamborough; they do not follow the vast shoals that collect off the East Anglian coast in late autumn. In the spring they turn up again with great regularity on the spawning grounds, where they are the objective of intensive fishing. These broad movements of the large cod can be clearly traced by means of the detailed fishery statistics collected by the English and Scottish authorities.

The eggs are of course pelagic, hatching after about 12 days. The larvae are about 4 mm. in length on hatching, and during their pelagic life of some two months grow to about 25 mm. During this period they drift at the mercy of the currents, with the result that they are distributed generally over the area to the south and east of the Dogger Bank; some, however, settle down inshore on the Scottish coast. A close correlation between the drift of the eggs and larvae in 1924 and the known movements of the water in that year was established by the Lowestoft workers (Graham *et al.* 1926). Little is known about their habits and distribution in the early months of their life on the bottom, but it seems certain that during the first two years of their life they work their way from the sandy and muddy grounds of the south and east towards the rough grounds at either end of the Dogger Bank, where they first appear in the commercial catches. Until they become mature at four years old they do not appear to carry out extensive migrations, but their food habits gradually change, their tastes shifting over from a crustacean to a fish diet, and as this happens they probably become more mobile. When they mature they take part in the spawning migration already described. How they find their way to the spawning grounds is completely unknown. Further references to papers on the migration of cod in the North Sea will be found in Graham (1934).

The stock of cod in the North Sea is more or less self-contained, and independent of the great *Arcto-Norwegian stock* which is found in more northerly waters. This stock has its headquarters in the comparatively shallow plateau north of the European coast, bounded by Bear Island, Spitzbergen and Novaya Zemlya, where, particularly round Bear Island and in the Barentz Sea proper, it is the object of a great trawl fishery prosecuted by the British and the Germans. The cod spend their early years in this area, which is their great feeding ground; here they fatten on euphausiids, decapod crustaceans and lamellibranchs, and pursue the shoals of capelan (*Mallotus*); their movements during this period are determined by their search for food. When they mature at about eight years old they carry out extensive spawning migrations to the west coast of Norway, concentrating in specially favoured areas in enormous spawning shoals. The most important and most famous of these concentrations is found inside the Lofoten Islands, where from the middle of January to the end of April there takes place in a very small area a great fishery, with lines, by small Norwegian vessels (Sund, 1931). The yield varies considerably, but averages about 20 million large cod. The fish are found in a layer of water of 4–5° C., generally at

some distance off the bottom. The occurrence of the cod in this intermediate layer has recently been demonstrated by means of the Husun echo-sounding apparatus (Sund, 1935 *a*). It is of interest to note that the cod, which in their northern zone of distribution live in water down to 2° C. or even less, seek somewhat warmer water for reproduction.

The eggs, which are produced in astronomical numbers, and the larvae, are distributed by the pronounced north-going current to the northern shores of Norway and particularly to the shallow areas of the Barentz Sea, where they grow

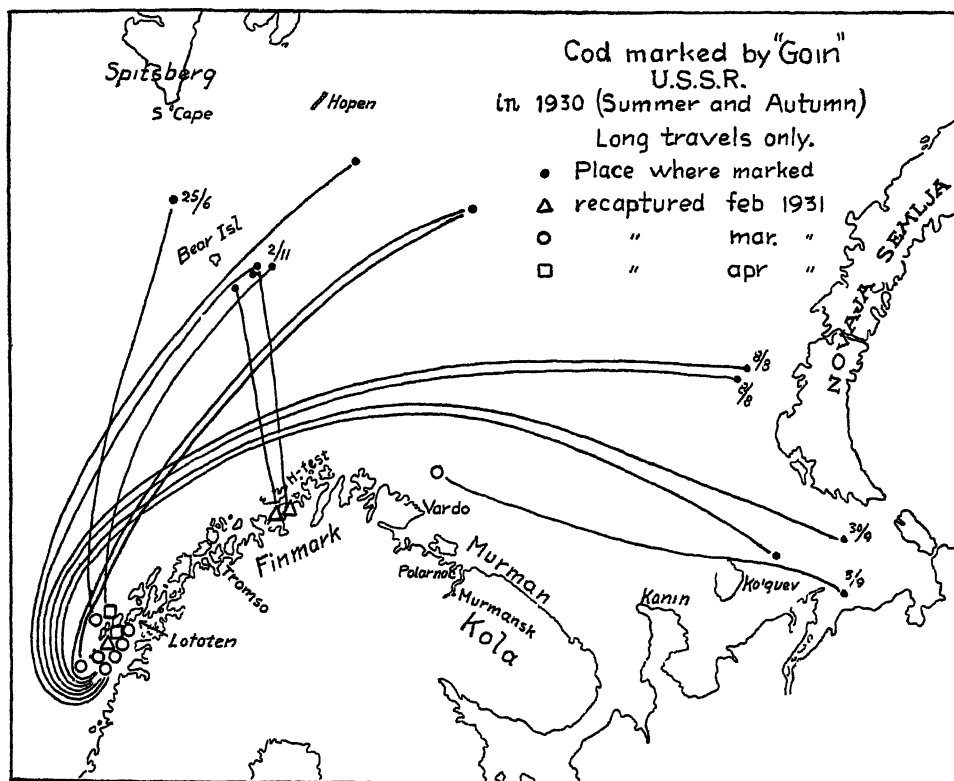


Fig. 1

up. The spent fish also travel towards the north, seeking the rich feeding grounds of the Barentz Sea and Bear Island. The occurrence of these extensive and well-marked migrations of the spawning and spent cod has been clearly demonstrated by the marking experiments carried out on a large scale by Hjort and his collaborators (Hjort, 1914, 1926; Iversen, 1934) and also of late years by Russian workers (Messiatzeva, 1932). The main results of the Russian work are shown in Fig. 1.

These broad movements conform to the general rule of a contranant spawning migration, and a denatant distribution of spent fish and eggs and larvae. It has recently been shown by Sund (1935 *b*) that the northerly movement of the spent fish takes place at the rate of about half a mile a day, or slower than the north-going

current, so the movement is not a passive one. The same observer is inclined to doubt whether in their southerly spawning migration the cod head against the current, as they appear to travel at a depth of some 300–400 metres, where it is possible there is a south-going current. However this may be, it does not affect the main point, that the cod spawn up-current and the products of spawning are transported down-current.

The same general rule is true of the *Icelandic stock* of cod which appears to be separate from the Arcto-Norwegian stock. Round Iceland the general circulation of the waters, as judged by drift-bottle experiments (Tåning, 1931), is anti-cyclonic or clockwise. The cod spawn in the spring in the warmer waters to the south and west of Iceland, and the eggs and larvae are transported by the Irminger current in a northerly and easterly direction, most of them growing up in the colder waters of the north and east coasts (Schmidt, 1931). The spent fish disperse from the spawning grounds in a similar way, to collect again in the ensuing spring in the south-west.

Up to a few years ago it was an accepted belief that the stocks of cod in the North Sea, Norwegian and sub-Arctic waters, Iceland, the Faeroes, Greenland and Newfoundland, were separate and self-contained, and this is still in the main true. But during the last few years striking evidence has been obtained by the Danes of a close relation between the Icelandic stock and the cod in west Greenland; the story is one of great interest and must be told in some detail, following the accounts by Jensen & Hansen (1931), Tåning (1934*b* and 1937) and Hansen *et al.* (1935).

Historical records show that the cod occurs every year at west Greenland, but usually only in small numbers, which keep mainly to certain fjords in the south. At irregular intervals a rich fishing develops, lasting for a few years, during which the cod spread as far north as Disko Bay. One such period happened round about 1820, another in the years 1845–9, when the fishing was good enough to attract vessels from Great Britain. Since then, up to about ten years ago, the normal conditions of poor yield obtained. The year 1917 marks the beginning of a rich period, when the cod have increased in abundance and spread gradually up the west coast of Greenland as far as Disko Bay.

This rich period appears to be connected with a large-scale hydrographical change whereby warmer sea temperatures occurred at Greenland, Iceland, and probably round Bear Island and in the Barentz Sea. Correlated with this change in hydrographical conditions there has been since about 1924 a shift in the fish fauna of west Greenland, southern species like the haddock, coal-fish and herring moving north into these waters, where they were previously almost unknown, Arctic species like the capelan and the Greenland halibut retreating farther to the north. Similar changes in the fauna have been clearly demonstrated by Saemundsson (1934) in Icelandic waters, where for the past ten years or so sea temperatures, especially on the north coast, have been above normal. Reference may also be made to the general paper by Berg (1935).

Marking experiments on Greenland cod were started in 1924 and repeated yearly until by 1934 a total of nearly 8000 fish had been marked. Up to 1929 the majority

of the cod recaptured were taken in Greenland waters, but a few turned up at Iceland on the spawning grounds. From 1929 onwards the number of emigrants from Greenland increased rapidly, being especially great in 1933 and 1934. In 1933, for instance, 72 per cent of the recaptured cod were taken in Iceland, against only 28 per cent in Greenland. The migration to Iceland had reached great dimensions. These migrating cod are mature fish; they move off in late autumn and winter and are caught in the spring on the spawning grounds on the south and west coasts of Iceland; they are therefore carrying out a spawning migration. Their route is not known with certainty, but it is probable they move up the east coast of Greenland and cross to Iceland by a submarine ridge known to exist north of 65° N. lat. The fish are mostly 9–11 years old, and belong to the rich year classes of 1922 and 1924. Most of them come from the southern districts of west Greenland, but migration to Iceland from east Greenland has also been demonstrated.

It has also been shown that cod may migrate from the spawning grounds at Iceland to the waters of west Greenland. Of sixteen long-distance migrants from Iceland recaptured in 1930–33, twelve had reached west Greenland, one half-way across to Greenland, one got as far as Newfoundland (over 2000 miles) and two reached the Faeroes (Fig. 2). There are also two records from Norway.

With regard to the origin of these cod migrating from Iceland Tåning writes as follows (1934*b*, p. 8): "It is not impossible that these cod have arrived from Greenland (or some other area) in the same year, prior to the spawning period in which they were marked, and thus they return to their original home waters immediately after spawning. . . . Probably, however, a part of the West-Greenland stock of cod originates from Icelandic spawning places, as investigations of the *Dana* in the years 1931–3 have shown a periodic drift of pelagic fry across the Denmark Strait to East-Greenland with the west-going branch of the Irminger Current."

Though the full story is not yet known, it seems probable that the increase of cod in Greenland waters is due to colonization from Iceland, both through the transport of fry by the west-going current and through active immigration of foraging parties of cod seeking food. The great abundance of cod in Iceland may have forced some to seek food farther afield.

There is a certain amount of spawning in Greenland waters, but apparently in these recent years of abundance the bulk of the mature fish have migrated for spawning purposes to the grounds at Iceland, which is the great centre for spawning for all the cod in this north-western region.

III. THE PLAICE

The broad migratory movements of the plaice in the southern half of the North Sea were elucidated in the early years of this century by the co-operative work initiated by the International Council for the Exploration of the Sea. There is a spawning migration of the mature fish in winter southwards towards a spawning area in the deepish water between the coasts of East Anglia and Holland. Here, especially in the winter months, a tongue of relatively warm and salt water extends up from the English Channel, and in this water the plaice spawn. The prevailing

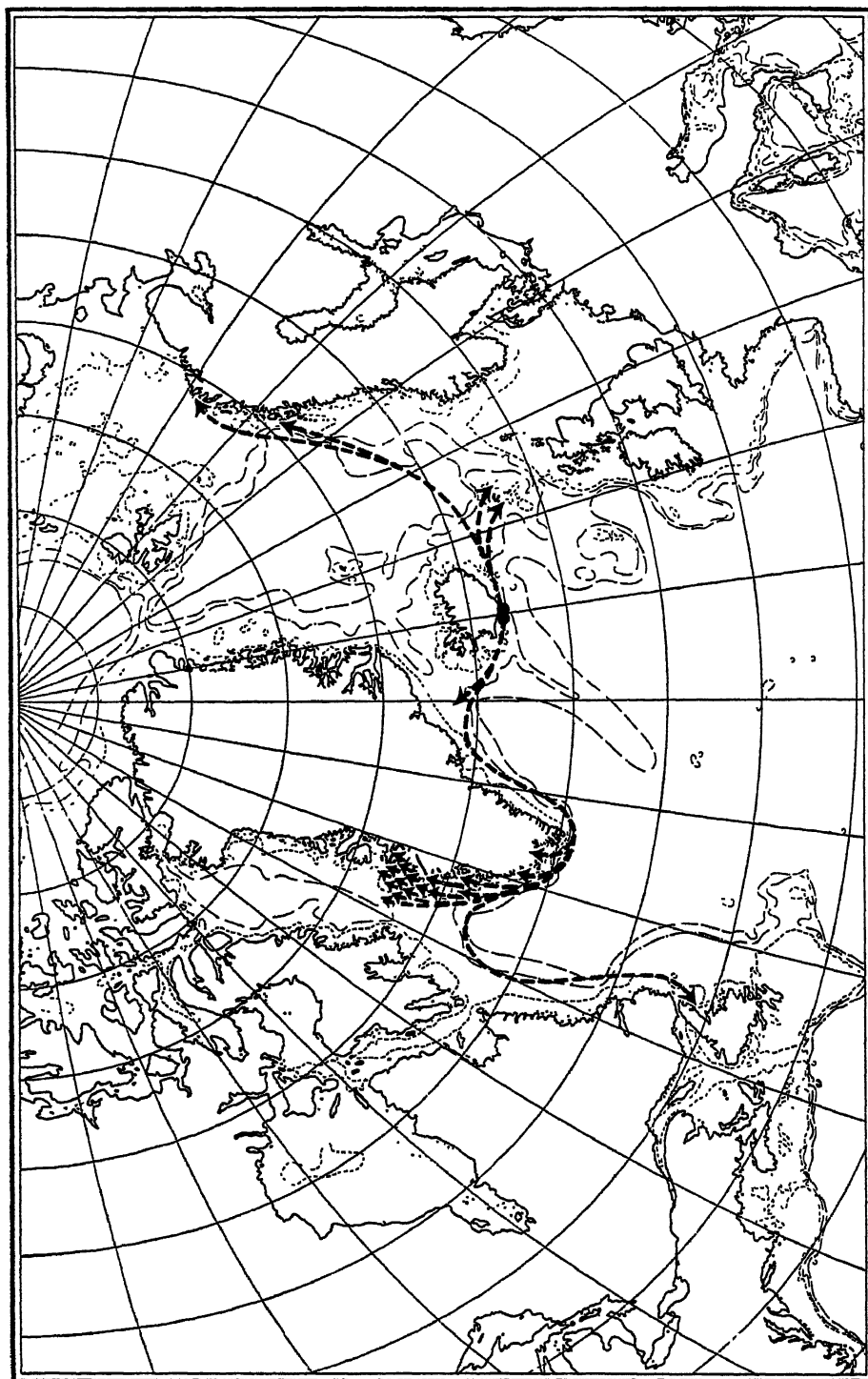


Fig. 2. Long-distance migrations of cod from the spawning grounds at Iceland. (By courtesy of Dr Tåning.)

current runs in a north-easterly direction and transports the eggs and larvae along the continental coast, where the young fish settle down in the shallow and sandy inshore waters. There is a return migration or dispersal of the spent fish in a general northerly direction. These movements are shown in a diagrammatic fashion in Fig. 3. We note that the spawning migration is in the main up-current or contranantant, and the post-spawning dispersal down-current or denatant. Also the spawning area is so situated that in normal circumstances the eggs and larvae are transported towards regions which are suitable for the development and growth of the young fish.

Another well-defined migratory cycle exists among the plaice which inhabit the waters off the east coast of Scotland. It has been shown by the Scottish investigators that these plaice, when they become mature, migrate northwards to a spawning area in the Moray Firth, collecting there from as far south as the Firth of Forth. This spawning migration also takes place against the prevailing current, which runs in a southerly direction some little distance off the east coast.

The existence of a third migratory cycle has been demonstrated by Bowman (1933) in the waters round the Shetland Islands. The plaice, being a fish frequenting water of moderate depth, is here confined to an inshore zone where the depth is less than about 50 fathoms, and the area is thus in the main a self-contained one so far as the plaice are concerned. There is a clockwise circulation of water round the islands, which carries with it the eggs and larvae produced in the spawning season. It has been shown by Bowman that the movement of the mature fish which are ready to spawn is counter-clockwise, or against the current.

A very interesting experiment was carried out by Bowman in this area. Over a period of years he transplanted some 2000 immature plaice from St Andrew's Bay, on the east coast of Scotland, to Shetland waters. He found that when these plaice became mature they joined in the contranantant spawning migration of the local fish, moving counter-clockwise, north about, round the islands. They did not attempt to get back to their ancestral spawning ground in the Moray Firth. It looks as if, when they became mature, they simply headed against the prevailing current, just as they would have done if left on the east coast. This experiment suggests that the spawning migrations of plaice may be to some extent accounted for by the fact that as the gonads mature the fish become sensitive to currents and move against them. An interesting parallel is provided by the observations of Beauchamp (1933) on *Planaria alpina*. This little flatworm becomes positively rheotactic as it develops sexually, and carries out accordingly an upstream spawning migration.

Experiments are at present being undertaken by the English and Scottish Fishery Departments in transplanting maturing plaice from the area of the southern North Sea migration cycle to that of the Scottish east coast cycle and vice versa, which will, it is hoped, throw further light upon the role of currents in the spawning migration.

Both plaice and cod, being bottom-living fish, may be assumed to be capable of showing so-called rheotaxis. It is, however, by no means certain that a simple positive rheotaxis occurring as the gonads mature is a sufficient explanation of the spawning migration in all cases. It does not seem to cover completely the case of the spawning

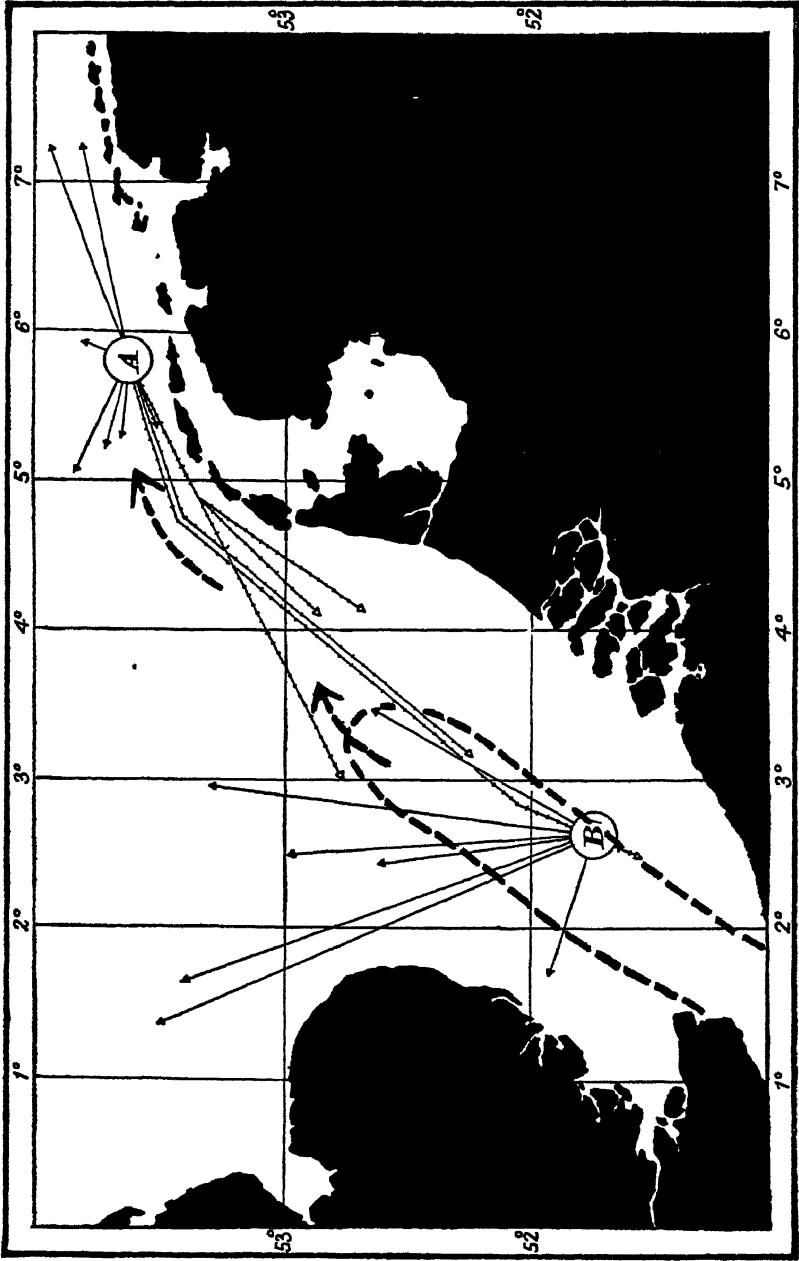


Fig. 3. Spawning migration and post-spawning dispersal of plaice in the southern North Sea as shown by marking experiments carried out at A and B.
(Reproduced by permission of Edward Arnold & Co. from Russell's *Behaviour of Animals*)

migrations of plaice in Icelandic waters. The plaice, like the cod, spawn in the warmer water off the south and west coasts, and many of the young fish live and grow up in this area. A proportion of the eggs and larvae are, however, carried by the clockwise circulation of the currents to the colder waters of the east coast, where they spend the first few years of their life. When they mature they migrate in the autumn to the south and west coasts to spawn, travelling in some cases as much as 300 miles. If the direction of the prevailing current were the sole guide to this migration, one would expect them to head against it round the north coast—and some of the plaice do take this route. But it has been clearly demonstrated (Tåning, 1934*a*) that many take the southerly route, travelling *with* the current (Fig. 4). As the plaice are limited in their distribution to a moderate depth, they do not leave the coastal banks, so that any continued movement of dispersal away from the east coast grounds, as the urge to migrate arises, must bring them in time to the spawning grounds in the warmer water of the south and west coasts. This is a possible, but not entirely satisfactory, explanation of the spawning migrations in Icelandic waters.

It is convenient to refer here to the investigations of Bowman (1935*a, b*) on the spawning migrations of the lemon sole (*Pleuronectes microcephalus* Day, now known as *Microstomus kitt* (Walbaum)). These are definitely contranatal, and afford an interesting parallel to the movements of the plaice in Scottish waters. In the years 1919–31 nearly 8,000 were successfully marked and over 1000 recaptured, of which 218 showed a definite spawning migration. The trend of the migratory movements carried out by ripening fish is northwards along the east coast of Scotland, from as far south as the Firth of Forth, into the Moray Firth. Some of these migrants go farther still and may reach Fair Isle or even the Shetlands. This route may also be taken by migrants from the Moray Firth itself, or they may leave the North Sea by the passage between the Orkneys and Fair Isle and move in a south westerly direction towards Cape Wrath and the Butt of Lewis. Bowman shows in detail that all these movements take place against the prevailing current.

IV. THE SALMON

An immense amount of work has been done on the migrations of the salmon, both in Europe and in North America, where on the Pacific coast the North Atlantic species *Salmo salar* is replaced by various species of *Oncorhynchus*, a closely allied genus. The general outlines of the story are well known and hardly need recapitulation here. The eggs are laid in the bed of a river, generally in its head waters or in a tributary, in shallow depths. The young inhabit the parent stream for varying lengths of time—two years on the average in *Salmo salar*—then migrate down to the sea, where they spend two or more years, feeding lustily and growing at a great rate. On the approach of maturity they return to the river in which they were born, or sometimes to an adjacent river, and ascend with great determination to the upper waters in preparation for spawning. They may return to the river long before the spawning season and while their gonads are little developed, or they may ascend late in the year with well-developed reproductive organs; there is often a spring run and an autumn run. The extensive literature up to 1928 is summarized and discussed

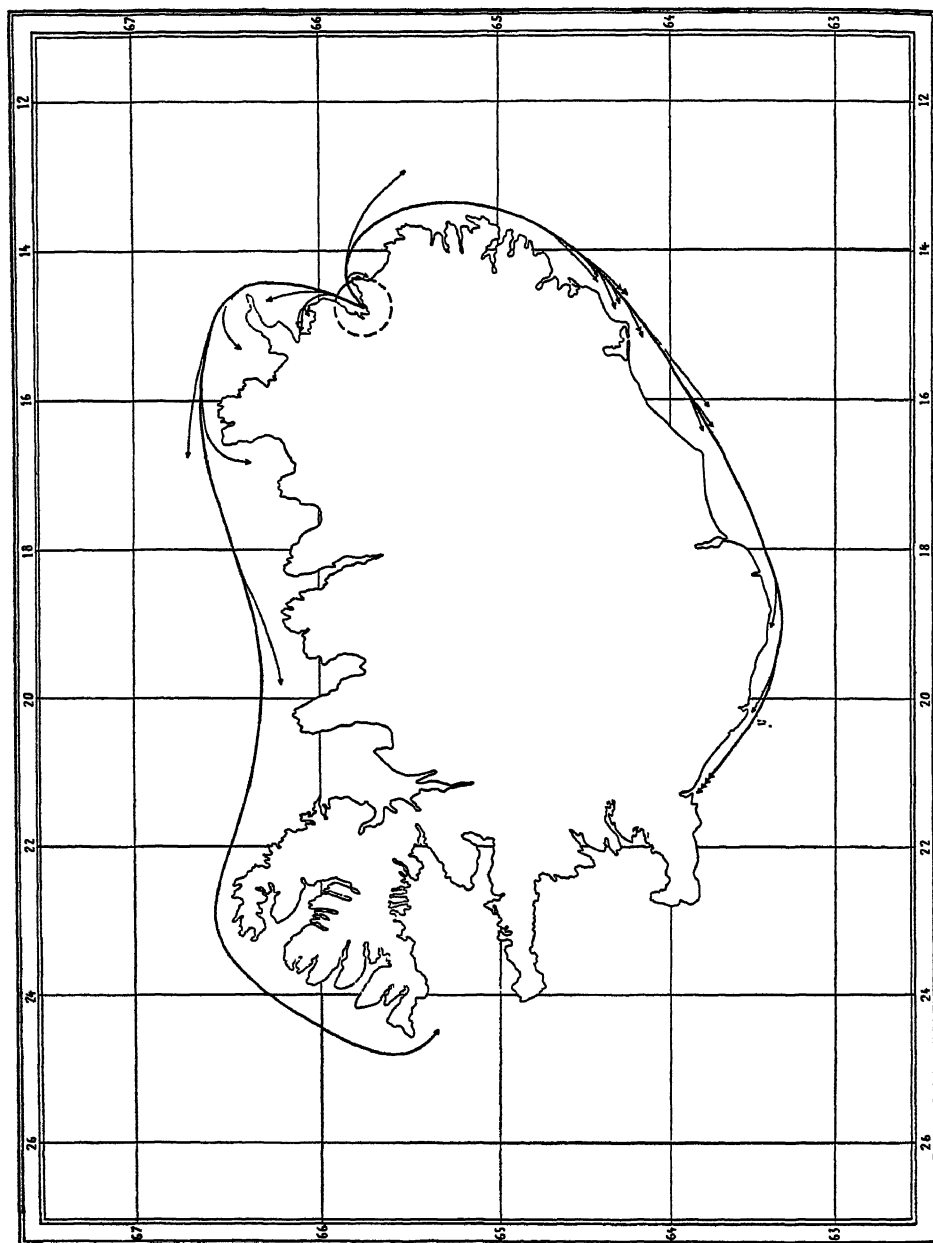


Fig. 4. Spawning migrations of plaice in Icelandic waters. (After Tåning)

in Scheuring (1929), and I propose to deal with one point only, on which light has been thrown by recent American work, namely the so-called "homing instinct".

There is a good deal of evidence, both direct and indirect, that salmon return as a general rule to their home river, sometimes even to the same spot in which they started life (see for instance Dahl & Sömmе, 1935). Is this due to a hereditary "instinct" to return always to the same river, or is it dependent upon individual experience? This interesting question could be tested by transplanting ova from the river in which they were laid into some other river and bringing them up there, and then finding out whether, after their sojourn in the sea, the fish returned to the river of birth or to the river of upbringing. Experiments of such a kind have been successfully carried out by Rich & Holmes (1929) and also by Snyder (1931), and they show that the salmon return to the river in which they have spent their early life, and not to the river in which they were spawned. While therefore the general impulse to return to fresh water at the onset of maturity is undoubtedly hereditary, there is no inherited tendency to return to the ancestral home. Return to the place of upbringing must be due to individually acquired experience, individual memory, and not to a problematic "inherited" or "ancestral" memory. This is a result of great general importance, and tells strongly against the theory that acquired experience can be transmitted to descendants and become hereditary. For if homing is exact, the salmon spawning in a particular river must have spawned there for many generations, giving every opportunity for the formation of an "ancestral habit" of returning to that particular river. But such a habit is in fact not formed.

In view of the significance of this conclusion it becomes of interest to examine the evidence in some detail. The very extensive experiments of Rich & Holmes were carried out in the Columbia River system on the chinook or king salmon (*Oncorhynchus tshawytscha*). The Lower Columbia River, some 90 miles long, receives the waters of two quite distinct systems, the Columbia River coming from the east, and the Willamette River flowing from the south, both with numerous tributaries. The salmon frequenting the Willamette system are "spring run" fish, those of the Columbia "autumn run" fish. The general scheme of the experiments was to transfer batches of eggs from some locality on the Willamette system to a station on the Columbia river system and to hatch and rear them there. They were liberated as fingerlings in 7-18 months, being marked in some distinctive way by amputation of fins. Those that were recaptured on their return from the sea, mostly as four- and five-year-old fish, ran in the spring, but returned to the Columbia River system, none of them entering the Willamette River. Their homing was, however, not so exact as that of the native Columbia River fish, possibly because the conditions in this river are not suitable for spring running fish. Thus in Exp. 7, out of 65,000 liberated at an age of 13 months, 252 were recaptured, mostly in the month of May, 2½-4½ years later. About half of these were taken above the creek of liberation and only three actually in the creek. Contrast the results obtained in a marking experiment with native Columbia River fish; 99 of the 504 recaptured were caught at the hatcheries where they were reared and liberated, five were caught in nearby tributaries, and the rest on the way up. "The most striking instance of this return

to the home stream is that of Spring Creek. This stream is so extremely small that it is difficult to see how the salmon could find it at all, and yet 82 of the fish marked here were recaptured here as adults, while only 4 were taken in other spawning tributaries" (1929, p. 263).

But the broad fact remains that all the fish from the transplanted eggs returned to the river in which they were brought up, and not to the river in which they were spawned. They retained, however, their hereditary "habit" of migrating up the river in spring, instead of in autumn as the native fish do.

Similar results have been obtained by Snyder (1931), who found that king salmon from eggs taken in the Sacramento River and reared in Klamath River return to the latter. He adduces evidence that accuracy of return is dependent upon the time spent in the rearing tributary. The results of experiments carried out in various creeks of the Klamath lead him to conclude that "when yearlings are introduced into, and given a sufficient exposure to the waters of a particular tributary, they tend on their return migration to seek out and enter that tributary, while under other conditions they may scatter to a considerable extent" (p. 76).

The conclusion seems justified from all this work that the young salmon in fresh water get to know their home waters and recognize them on their return. They must remember the way back to their home river after their extensive journeyings in the sea. How they do this we do not yet know.

Cod and plaice on the one hand, salmon on the other, are typical of two different cases. In the first, with free drift of eggs and larvae it seems quite impossible for the young to have any knowledge or memory of their spawning places; their return to these areas appears to be due to their response, on approaching maturity, to simple environmental cues, of which one is probably current. In the second set of cases, exemplified by the salmon, the young have an opportunity, during their sojourn of one or two years near the place of their birth, of becoming acquainted with it, and, as we have seen, there is evidence that after their migration to the sea they do remember the way back and retain a topographical memory of the river in which they were brought up. Possibly in their return from the open sea they are guided not entirely by topographical memory, but also by simple perceptual clues, of temperature, current and so on, but the exact homing to a particular river and to a particular tributary appears definitely to be due to individual experience, individual memory.

V. SOME KATADROMOUS SPECIES

In contrast to the salmon and other anadromous forms, which run up into fresh water to breed (showing thus a general analogy with the contranatal spawning migration and the denatal post-spawning dispersal of most marine fishes), there are of course a number of species which live in fresh or estuarine waters and seek the sea for the purpose of breeding. A case in point is the flounder (*Pleuronectes flesus* Day, now known as *Platichthys flesus* (L.)), which is predominantly an estuarine fish, but migrates out for spawning to the open sea.

Then there is the classical case of the European eel (*Anguilla anguilla* (L.)), which spends its adult life in fresh waters, migrating when mature thousands of miles into

the Atlantic to spawn in deep water in the Sargasso Sea. The story as elucidated by the masterly researches of the late Dr Johannes Schmidt is too well known to need recapitulation here. We may note, however, that the dispersal of the larval forms or leptocephali is, so far as we know, a passive one, a drift with the currents easterly across the North Atlantic, lasting about three years. But on this denatant and passive dispersal there follows, when they reach the European coasts, an active contranant migration of the glass eels and elvers up our rivers and streams.

An admirable summary of Schmidt's researches on the various species of eel both in the Atlantic and the Pacific has recently been published by the Carlsberg Foundation (1935). Without going into details about the Indo-Pacific stocks, we may mention one very interesting result obtained on the *Dana's* cruise round the world in 1928-30. Schmidt found that the four species of eel which inhabit Sumatra and the western part of Indo-Malaya breed in a deep trough, known as the Mentawai Deep, lying some little distance off the west coast of Sumatra. The eels migrating from fresh water in this area have only a short distance to go to find water of suitable depth (5000 metres) and salinity for spawning; they breed "just outside the door", and the larvae have no great distance to travel back to the coast; they are more rapid in their development and do not reach so large a size as the leptocephali of the North Atlantic which have such a long journey to perform.

I shall give one more case of a katadromous spawning migration, that described recently by Whitley (1935) for the New Zealand "whitebait". This fish has nothing to do with our British whitebait, which is a mixture of young herring and young sprats, but belongs to the mainly fresh-water family of the Galaxiidae, which is confined to the southern hemisphere. Most of the species breed in fresh water, but some descend to the sea for spawning, and among them is the "whitebait", *Austrocobitis attenuatus*. The adult fish run down to tidal waters in the spring in countless myriads, collecting close to the banks wherever there are rushes or other vegetation. They attach their eggs to this vegetation, quite close to the surface, at high water. Whitley quotes Captain Hayes to the effect that "Spawning does not take place until the highest of the spring tides has passed. The ova are thus left high and dry when the tide recedes, and since they are deposited as near the water's edge as the fish can get, and the tides which follow are of diminishing size, there can be no further contact with the water until the occurrence of next spring tides—at the earliest a fortnight later. . . . It has been found that if the spring tides succeeding those during which the spawning took place do not reach the zone where the spawn is deposited, the eggs remain unhatched until a tide sufficiently high to reach them occurs."

The young fish, soon after hatching, migrate *en masse* up the river, struggling against the stream, just as elvers do.

VI. TIDAL SPAWNING; THE CASE OF *LEURESTHES*

The association of spawning with spring tides, which is indicated in *Austrocobitis*, has been clearly demonstrated in the Californian grunion or sand smelt, *Leuresthes tenuis*, by W. F. Thompson (1919) and by Clark (1925). This is a purely marine

species, and moves in from the sea to bury its eggs in the sand near high-water mark. In the months of March to July, on the long sandy beaches of California, shoals of these little fish appear at the water's edge at the top of the tide, during 2-3 nights after the night of highest springs. The spawning run does not begin till high water, and continues during the first of the ebb, for about 1 hour. The females bury themselves tail first in the soft sand to the level of the pectoral fins, and deposit their eggs in a mass or "pod" some 2 in. under the surface of the sand; one or more males are in attendance on each female and fertilize the eggs. When the tide recedes, it is found that the eggs now lie deeper—on an average 4 in. down; this is because the eggs have been laid near the average limit of wave action, in a zone of deposit of sand. If they were laid a little further down the beach they would lie in a zone of erosion and would be quickly swept away by wave action.

As the eggs are deposited shortly *after* spring tide, during a series of diminishing tides, they remain undisturbed until the next springs, when the sand in which they lie is eroded by the advancing tides and the eggs washed out. They hatch as soon as they are washed out, and they will not hatch until freed from the sand. If by mischance they do not get washed out a fortnight after deposit, a fair proportion of them can survive under the sand till the next springs, a month's time in all. The timing of the spawning is amazingly well adapted towards ensuring an undisturbed period of 14 days under the sand and the high probability of release by the waves when this period is completed.

When Thompson first described the phenomenon, the evidence pointed to spawning taking place only after full-moon springs. It has since been shown by Clark that spawning occurs also after new-moon springs—it is a tidal phenomenon rather than a full-moon one. There is a definite organic rhythm in the female, leading to the ripening of a fresh batch of eggs every 14-15 days. "As soon as one batch of eggs matures and is spawned out, another batch begins to develop, is matured, and spawned out two weeks later. Thus, after an individual fish starts spawning it continues to spawn periodically on each series of high tides throughout the breeding season" (Clark, 1925, p. 39).

VII. THE ANCHOVY AND THE ZUIDER ZEE

A very interesting account has been given recently by Fage (1935) of the effect upon the anchovy of the reclamation of the Zuider Zee. As is well known, this area was a favourite spawning and nursery ground for the local race of the anchovy which inhabits the southern part of the North Sea and the English Channel. In April and May shoals of mature fish appeared at the mouth of the Scheldt and in the entrance to the Zuider Zee; a few went east to the Dollart. The chief spawning centre lay in the Zuider Zee, between Enkhuisen and Wieringen, where in May to July the fish found the high temperatures, 17-18° C., necessary for their spawning—the species is a southern one, and is here at the northerly limit of its distribution. After spawning, the shoals rapidly left the coast of Holland and dispersed in the open sea. Not only were the temperature conditions for spawning peculiarly

suitable in the Zuider Zee, but the rich production of phytoplankton was extremely favourable for the rapid development of the larvae and young.

Access to the normal spawning grounds in the Zuider Zee was progressively cut off from 1924 onwards, as the work of reclamation proceeded, and by 1930, when the great dike was three parts completed, the bulk of the fish were forced to find other grounds. They spread eastwards to the estuaries of the Weser and the Elbe and to Jade Bay. In subsequent years some even penetrated into the Baltic, not, however, as spawning shoals. Now that a great part of the Zuider Zee has been completely cut off, it appears that the anchovy spawns in the estuary of the Scheldt and in the Waddensee—that is to say, near its old spawning grounds—and also farther east, in the estuaries of the Ems, the Weser and the Elbe, and possibly off the Holstein coast. These grounds are not so favourable for the growth of the young, but in Fage's opinion the species will be able to adapt itself to the new conditions. The case is interesting as showing what a species can do if its normal spawning areas are cut off.

VIII. SUMMARY

A knowledge of the migratory movements of fish is of great practical importance in the commercial fisheries, and accordingly much research has been carried out on the subject by the maritime nations of Europe and by the United States, largely by means of marking experiments. The general schema of the migrations, which holds good for most species, is (1) a spawning migration of mature or maturing individuals towards a definite spawning area, generally upstream or up-current, (2) a dispersion of the spent fish, generally downstream or down-current, in search of food, leading sometimes to definite feeding migrations. The general effect of this cycle of migration is to keep the species within its ecological norm.

These principles are illustrated by reference to the cod and the plaice in the North Sea, Norwegian coast and Barentz Sea, Iceland and Greenland. It is shown that in each of these areas there is a definite migration cycle, and evidence is brought forward that in most cases the spawning migration takes place against the prevailing current. The recent spread of cod from Iceland to Greenland and their return to the Icelandic spawning grounds is described in some detail.

In cod and plaice the eggs and larvae are pelagic and are at first distributed passively by the currents. It seems impossible that they should retain any individual memory of their spawning places. In the case of the salmon, however, the eggs are deposited in the upper waters of rivers and streams and the young fish spend one or more years of their life in the vicinity. It has been shown for the chinook salmon of the Pacific that the fish returning from the sea to spawn remember and return to the stream in which they were brought up, even though they had been spawned in a stream hundreds of miles away. Hence individual and not ancestral memory is the dominant factor in the so-called "homing instinct".

To provide a contrast with the salmon and other anadromous forms, which run up rivers to spawn, brief reference is made to katadromous forms such as the flounder, the eel, and the New Zealand "whitebait" (*Austrocobitis*). The last-named

appears to spawn just after the height of spring tides, as is also the case with the Californian grunion (*Leuresthes*), whose very remarkable spawning habits are discussed in detail.

In conclusion, an account is given of the response of the local anchovy to the cutting off of its main spawning ground through the reclamation of the Zuider Zee; it has adopted new spawning grounds outside and has spread further north.

The article is not to be regarded as exhaustive, but merely as supplementary to the comprehensive surveys of the subject by Meek and Scheuring.

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DIE MESSUNG UND BEDEUTUNG DER ELEKTROLYTISCHEN POLARISATION IM NERVEN

VON H. LULLIES

(Köln)

(Received 10 May 1936)

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I. ALLGEMEINES

JEDES tierische und pflanzliche Gewebe zeigt eine gewisse Struktur. Es sind Grenzflächen vorhanden, an denen sich seine Eigenschaften sprunghaft ändern. Wichtigste Lebensvorgänge spielen sich an solchen Grenzflächen ab. Sie sind verantwortlich zu machen für das Auftreten von Aktionspotentialen bei Änderungen des Tätigkeitszustandes eines lebenden Gebildes, aber auch für alle diejenigen Erscheinungen, die man "passiv" beim Anlegen elektrischer Potentialdifferenzen an ein Gewebe beobachtet.

Hierbei treten Ionen- oder Ladungsverschiebungen und damit Störungen des Gleichgewichts an den Grenzflächen auf. Sobald die Störung ein gewisses Mass überschreitet, kann es zu weiteren selbständig ablaufenden Prozessen kommen, zu Vorgängen, die man als "Erregung" bezeichnet. Die Störung wirkt aber auch auf den hindurchgeleiteten Strom zurück. Es treten elektromotorische Gegenkräfte auf, das Gewebe zeigt *Polarisation*.

Diese Rückwirkung des Gewebes auf den elektrischen Strom, die Polarisation, wird abhängen von der Beschaffenheit seiner Grenzflächen und der angrenzenden Elektrolyte, aber auch von ihrer räumlichen Anordnung. So sind Messungen an den verschiedensten Objekten, vor allem an der *Haut* (Gildemeister, 1919, 1922, 1928 a, b; Lullies, 1928; Hozawa, 1928, 1931, 1932; Saito, 1931; Tsuji, 1932),

ferner an *Seeigeleiern* (Cole, 1928), *Blutkörperchen* (Fricke & Morse, 1925; McClendon, 1926) und am *Muskel* (u. a. Sapegno, 1930; Achelis, 1932; Bozler, 1935; Bozler & Cole, 1935) durchgeführt, um über das Verhalten ihrer Grenzflächen in dieser Hinsicht Aufschluss zu erhalten.

Auch am *Nerven* ermöglichen Messungen der Polarisation Aussagen über die Eigenschaften und die Anordnung seiner Grenzflächen (Lullies, 1930 *a, b*; Labes, 1932 *a, b*; Labes & Lullies, 1932 *a, b, c*). Darüber hinaus werden aber solche Messungen, gerade am Nerven, eine besondere Bedeutung haben für die viel diskutierte Frage nach dem Zusammenhang zwischen *Polarisation* und der gleichzeitig gemessenen *Reizwirkung* des elektrischen Stromes. In den "Zeitfaktoren", die die elektrische Reizung beherrschen (vgl. z. B. die kürzlich erschienene Darstellung von Hill, 1936), steckt jedenfalls neben den Zeitkonstanten von Gewebsprozessen der Zeitfaktor der Polarisation. Wenn man diesen direkt messen könnte, würde die Frage nach der Bedeutung der anderen Konstanten eine sicherere Grundlage erhalten.

Im Folgenden soll an einigen Beispielen gezeigt werden, wie man auf diesem noch wenig beschrittenen Wege in der Analyse der Nerveneigenschaften weiterzukommen versucht. Theoretische Erörterungen können hier umsomehr zurücktreten, als sie vorläufig noch zu keinem abschliessendem Ergebnis führen. Der Versuch einer Darstellung an dieser Stelle scheint trotzdem gerechtfertigt, weil über diese Dinge bisher nur kurz auf physiologischen Tagungen berichtet ist (Lullies, 1927, 1929 *a*, 1934 *a*), und die Methoden für die Analyse anderer biologischer Strukturen ebenfalls von Bedeutung sind.

II. ZUR METHODIK

Die Messung der Polarisation eines Gebildes erfolgt im Prinzip stets durch die Messung seiner Rückwirkung auf einen elektrischen Strom von bekanntem Verlauf (vgl. Lullies, 1932). Besonders bequem, auch für die theoretische Auswertung, ist die Messung mit sinusförmigen Wechselströmen in einer Wheatstone'schen Brücken-Schaltung, nach Methoden, die von Gildemeister (1919) in die Physiologie eingeführt sind. Wenn sich in einem Zweig der Brücke ein Nerv zwischen geeigneten Elektroden befindet, so speichert er an seinen polarisierbaren Grenzflächen Elektrizität. Man muss in den anderen Zweig vor einen Ohm'schen Vergleichswiderstand eine bestimmte Kapazität schalten, um den Strom im Nullzweig der Brücke verschwinden zu lassen. Die Grösse $1/\omega C$ (ω = Frequenz des Wechselstroms in 2π sec., C = Kapazität) nennt man bei einem solchen Leiter mit Kapazität den *Blindwiderstand* (reactance). Der scheinbare Ohm'sche Widerstand ist der sogenannte *Wirkwiderstand* (resistance) des Gebildes. Beide Grössen ändern sich gesetzmässig mit der Frequenz. Wenn man solche Messungen über einen möglichst grossen Frequenzbereich durchführt, so besitzt man alle Angaben über das Verhalten der Polarisation, die man sich überhaupt verschaffen kann, denn jede andere Stromform ist nach *Fourier* als Summe von Sinusschwingungen darstellbar.

Für Messungen am Nerven sind besonders bei höheren Frequenzen besondere

Vorsichtsmassregeln notwendig, vor allem sorgfältige Abschirmung und Symmetrierung der Brücke gegen die Erde. Von grösster Bedeutung ist die Ausführung der stromzuführenden *Elektroden*. Die Stromzuleitung muss durch "Flüssigkeits-elektroden" erfolgen, in die grosse platiniierte Platinbleche tauchen. Dabei sind die Elektrodengefässe durch geerdete metallische Schutzhüllen vollständig gegeneinander abgeschirmt. Der Nerv kommt, wie Fig. 1 zeigt, durch einen Schlitz in zwei Paraffingefässen aus einer senkrechten Flüssigkeitswand heraus, was für die rechnerische Auswertung der Ergebnisse von grossem Vorteil ist.

Sehr bequem ist in einer solchen Anordnung die gleichzeitige Bestimmung der *Schwellenstromstärke*: Wird der Messtrom allmählich verstärkt, so hört man im Telephon des Nullzweiges der Brücke das Einsetzen der Aktionsströme. Wenn man im Nullzweig der Brücke einen Kathodenstrahl-Oszillographen anwendet, ist die Messung im gesamten Frequenzgebiet besonders einfach durchzuführen.

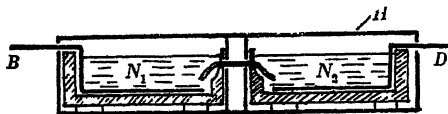


Fig. 1. Abgeschirmte Flüssigkeits-Elektroden für die Messung der Polarisation am Nerven. BD = Stromzuleitung, H = geerdete Blechhülle. (Lullies, 1930, 1932.)

Man hört oder registriert bei dieser Anordnung die Aktionsströme am Ort der Reizung. Bemerkenswert ist es, dass unter diesen Umständen, bei allmählicher Verstärkung der Mesströme der Nerv in vielen Fällen zuerst in rhythmische Tätigkeit gerät. Man findet Salven von Aktionsströmen, die durch Pausen voneinander getrennt sind. Die tätigen Fasern arbeiten oft zunächst synchron. Mit Zunahme der Stromstärke werden die Pausen kürzer, die Salven folgen rascher aufeinander, bis sie schliesslich zu einem kontinuierlichen Vorgang verschmelzen. Es handelt sich um ein Phänomen, das für unsere Vorstellungen von der Entstehung von Rhythmen im Nervensystem nicht ohne Interesse ist und genauer untersucht wird (Lullies, 1934 b).

III. POLARISATION UND SCHWELLENSTROMSTÄRKE IM MARKHALTIGEN NERVEN

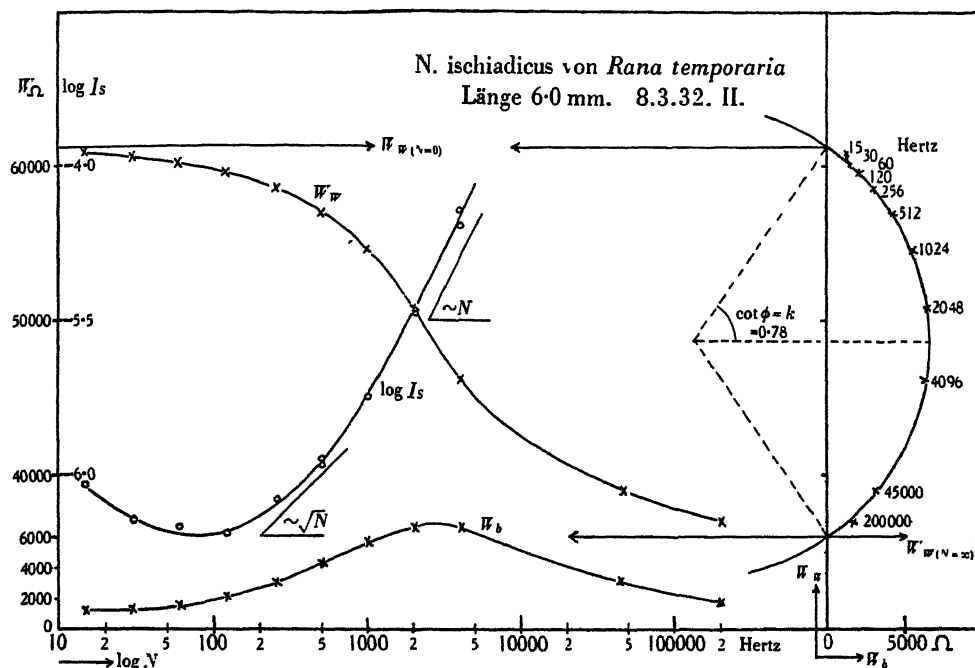
(1) Bei sinusförmigen Wechselströmen

Wenn man in dieser Weise Wirk- und Blindwiderstand eines Froschischiadicus bestimmt und gleichzeitig die Schwellenstromstärke misst, so ergibt sich sehr regelmässig ein Bild, wie es in Fig. 2 dargestellt ist.

Der *Wirkwiderstand* (W_w) nimmt von einem Minimum bei hohen Frequenzen (aufgetragen ist der Logarithmus der Frequenz) mit abnehmender Frequenz zu und nähert sich einem Maximum bei extrem niedrigen Frequenzen. Der *Blindwiderstand* (W_b) fällt zu beiden Seiten eines Maximums, das bei 2000–3000 Hertz (= Schwingungen p. Sec.) liegt, auf sehr kleine Werte ab. Die *Schwellenstromstärke* (I_s), deren Logarithmus aufgetragen ist, steigt, wie jetzt ja gut bekannt ist (Achelis,

1930; Coppée, 1934), bei hohen Frequenzen proportional der Frequenz, bei sehr hohen Frequenzen vielleicht noch rascher an. Bei mittleren Frequenzen nimmt sie proportional der Wurzel aus der Frequenz, dann langsamer ab und erreicht zwischen 60 und 120 Hertz ein Minimum, um mit weiter abnehmender Frequenz wieder anzusteigen.

Die Kurven der Fig. 2 beziehen sich auf ein Nervenstück von 6 mm. Länge. Wie sich die Widerstandsverhältnisse bei verschiedenen langen Nervenstrecken gestalten, ist von Lullies (1930) genauer untersucht und von Labes (1932 b) und



(2) Bei anderen Stromformen

Ganz entsprechende Ergebnisse haben, wie nicht anders zu erwarten, Messungen mit anderen Stromformen ergeben. Man kann aus den Widerstandskurven der Fig. 2 voraussagen, wie der Strom im Nerven beim Anlegen und Abschalten einer konstanten Spannung oder bei der Entladung eines Kondensators verlaufen muss.

So konnte mit Hilfe eines Elektrometers und eines Helmholtz'schen Pendels die sogenannte "Anfangszacke" bei einem Froschischadicus zum ersten Mal nachgewiesen und aufgezeichnet werden (Lullies, 1929b, 1930, 1932, S. 1277). Im

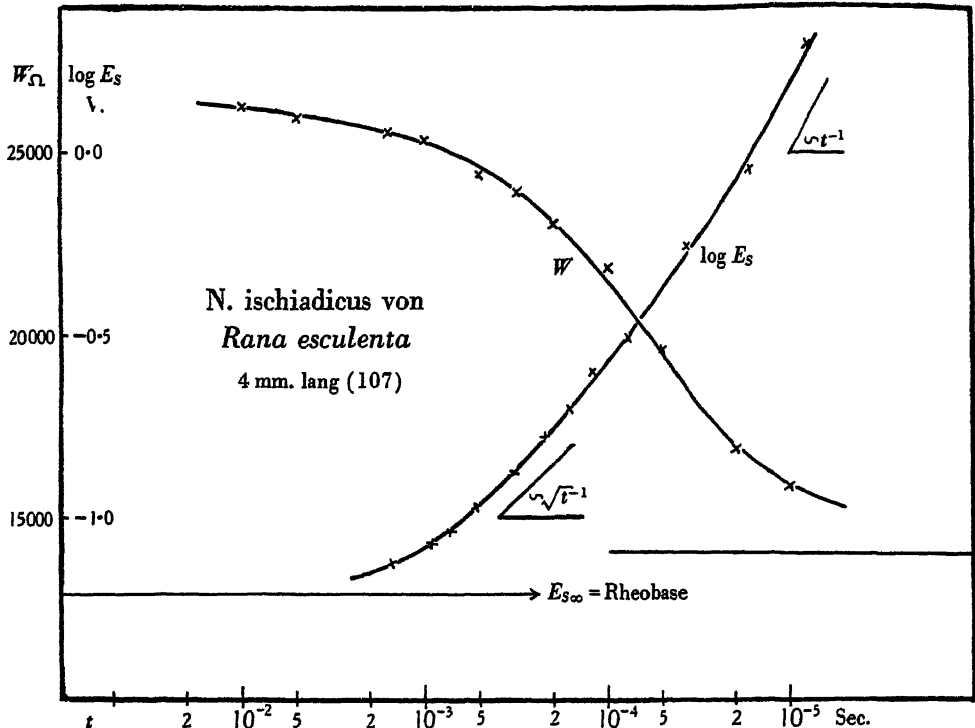


Fig. 3. Scheinbarer Widerstand W eines Froschischadicus zu verschiedenen Zeiten t nach Anlegen einer konstanten Spannung von 100 mV. und Schwellenspannung E_s für verschiedene Dauer t ihres Einwirkens. Abszisse: Log t (sec.). Ordinate: Scheinbarer Widerstand (Ohm), Logarithmen der Schwellenspannung (Volt).

ersten Augenblick des Anlegens einer konstanten Spannung an ein 4 mm. langes Nervenstück kann die Stromstärke nahezu doppelt so gross, der scheinbare Widerstand also halb so gross sein, als nach einigen zehntausendstel Sekunden. In Fig. 3 ist der scheinbare Widerstand (W) eines 4 mm. langen Froschischadicus dargestellt, wie er sich aus der Augenblicks-Stromstärke zu verschiedenen Zeiten nach Anlegen einer konstanten Spannung von 100 mV. ergibt. Ferner ist die Schwellenspannung (E_s) für verschieden lange Zeiten ihrer Einwirkung angegeben. Von "rechteckigen Stromstössen" kann man offenbar nicht sprechen. Die Ähnlichkeit mit den Ergebnissen der Wechselstrommessungen ist bei der gewählten Art der Darstellung sehr

deutlich. Das Verhalten von Widerstand und Reizschwelle bei verschiedenen *Streckenlängen* ist unter ähnlichen Bedingungen (für eine konstante Spannung von 0.8 msec. Dauer) von Rushton (1934) genauer untersucht.

Auch eine Kondensatorenentladung wird erwartungsgemäss deformiert, und auch diese Wirkungen können zahlenmässig ausgewertet werden (Lullies, 1927, 1932, S. 1259). Für die weitere Betrachtung sollen hier nur die Ergebnisse der Wechselstrommessungen herangezogen werden.

IV. DIE BEZIEHUNGEN ZWISCHEN DEN GEMESSENEN POLARISATIONSGRÖSSEN UND DER SCHWELLENSTROMSTÄRKE

Entscheidend für die Reizwirkung eines elektrischen Stromes können nach unseren heutigen Kenntnissen nur Konzentrations- oder Ladungsänderungen sein, die der Strom an polarisierbaren Grenzflächen des Nerven bewirkt. Diese sind aber auch für das Verhalten des Wirk- und Blindwiderstandes verantwortlich zu machen und müssten sich daher aus diesen Grössen ableiten lassen. Dabei setzt man zunächst voraus, dass ein solcher Zusammenhang überhaupt besteht, und dass diejenigen Grenzflächen und Ionen, die die gemessene Polarisation bestimmen, auch für die Vorgänge bei der Erregung entscheidend sind. Jedenfalls wird eine Prüfung dieser Voraussetzung möglich sein. Auch wenn die Beziehungen verwickelter sein sollten, kann eine Klärung der Frage nach dem Einfluss der Polarisation nur durch eine solche Betrachtung erfolgen. Dabei gelten die folgenden Sätze nicht nur für den Nerven, sondern ganz allgemein für das Verhalten der Polarisation aller Gewebe.

Die verschiedenartigen Verluste und ihr Einfluss auf die Polarisation

Massgebend für die Frequenzabhängigkeit der verschiedenen Grössen, des Wirk- und Blindwiderstandes und der Konzentrationsänderung, sind die "Verluste", die bei der Speicherung der hindurchgeschickten Elektrizitätsmengen an den Grenzflächen auftreten.

Diese Verluste können verschiedene Ursachen haben. Es ist

(1) die *Diffusion*, die die herangeführten Ionen zu zerstreuen sucht und zum Teil zerstreut. Diese Verluste berücksichtigt die Theorie von Nernst (1908);

(2) können unpolarisierbare *Nebenschlüsse* im Gewebe den Strom an den polarisierbaren Grenzflächen vorbeiführen. Die Wirkung solcher Nebenschlüsse lässt sich relativ einfach übersehen (vgl. z. B. Lullies, 1932; Cole, 1932);

(3) kann ein sehr geringer Abstand der polarisierbaren Grenzflächen voneinander, der von Hill (1910) zuerst in Betracht gezogen ist, zu einem Ausgleich von Konzentrationsdifferenzen führen, im Folgenden kurz als "*Hill'sche Schicht*" bezeichnet;

(4) schliesslich führt die Kernleiter-Struktur des Nerven zu Verlusten besonderer Art, *Kernleiter-Verlusten*, die von Labes (1932 a, b) zuerst in sehr vollständiger Weise behandelt sind (Labes & Lullies, 1932 b).

Die Theorie lässt für alle diese Fälle, unter bestimmten Voraussetzungen, die Polarisation bei verschiedenen Frequenzen und damit die zu erwartende Frequenzabhängigkeit von Wirk- und Blindwiderstand berechnen. Der Vergleich mit den Messungsergebnissen würde dann angeben lassen, welcher Art die Verluste im Nerven sind, und welche Veränderungen ein bestimmter Strom an den Grenzflächen bewirkt. Besonders anschaulich werden die Verhältnisse wenn man wie in Fig. 4 den Wirkwiderstand als Funktion des Blindwiderstandes graphisch darstellt.

1. Bei Verlusten, die nur durch *Diffusion* bedingt sind, ergeben sich hierbei gerade Linien. Ihre Neigung gegen die Abszisse ist verschieden, je nach dem Betrage dieser Verluste. Bei freier Diffusion in einer Dimension ist der Tangens des Neigungswinkels der Geraden, des sogenannten Verlustwinkels, gleich 1. Ist ein Teil der Ionen in Doppelschichten fixiert, so wird der Winkel kleiner. Bei reiner Doppelschichtenkapazität wäre er gleich 0 (Krüger, F., 1903). Im *Kernleiter*, in dem die Diffusionsverluste in der Längsdimension hinzukommen (Labes, 1932 b), wird der Winkel entsprechend grösser.

2. Beim Vorhandensein eines Nebenschlusses wird aus der Geraden das Stück eines Kreises, dem man den ursprünglichen Verlustwinkel der Polarisationskapazität und die Grösse des Nebenschlusses entnehmen kann. Cole (1932) wies darauf hin, dass sich bei den meisten Geweben sehr annähernd eine derartige Kreiskurve ergibt. Für die genauere Analyse ihrer Struktur werden jedoch gerade die Abweichungen von der Kreiskurve, die bei höchsten und bei niedrigsten Frequenzen auftreten von besonderer Bedeutung sein. Im *Kernleiter* ist der Kreis deformiert. Bemerkenswert ist aber, dass ein Ohm'scher Übergangswiderstand der Kernleiter-Membran die Kurve wieder so beeinflussen würde, dass sie von einem Kreis praktisch kaum zu unterscheiden ist (Labes, 1932 b).

3. In den letzten Diagrammen der Fig. 4 ist das Verhalten der beiden Grössen beim Wirksamwerden einer "Hill'schen Schicht" (Konzentrationsänderung 0 im Abstände $a/2$ von der Grenzfläche (Hill, 1910)) dargestellt, oben bei einem einfach querdurchströmten Gebilde, unten im Kernleiter. Die Kurven zeigen nur bei hohen und niedrigen Frequenzen charakteristische Abweichungen von der Kreisform.

Anwendung auf den Nerven

Wie man sieht (Fig. 2) ist beim Nerven des Frosches die W_w/W_b -Kurve mit grosser Annäherung über einen grossen Frequenzbereich ein Kreisabschnitt. Der Froschnerv verhält sich also wie ein polarisierbares Gebilde mit Verlusten durch Nebenschlüsse (Fig. 4). Vielleicht kommt dieses Verhalten durch das Zusammenwirken verschiedenartiger Umstände zustande, z. B. von Kernleiterverlusten mit Übergangswiderstand. Auch die Möglichkeit einer "Hill'schen Schicht", unter Umständen mit Kernleiterverlusten wäre in Betracht zu ziehen. Für eine Entscheidung zwischen den verschiedenen Möglichkeiten haben eine besondere Bedeutung die Messungen mit besonders hohen und besonders niedrigen Fre-

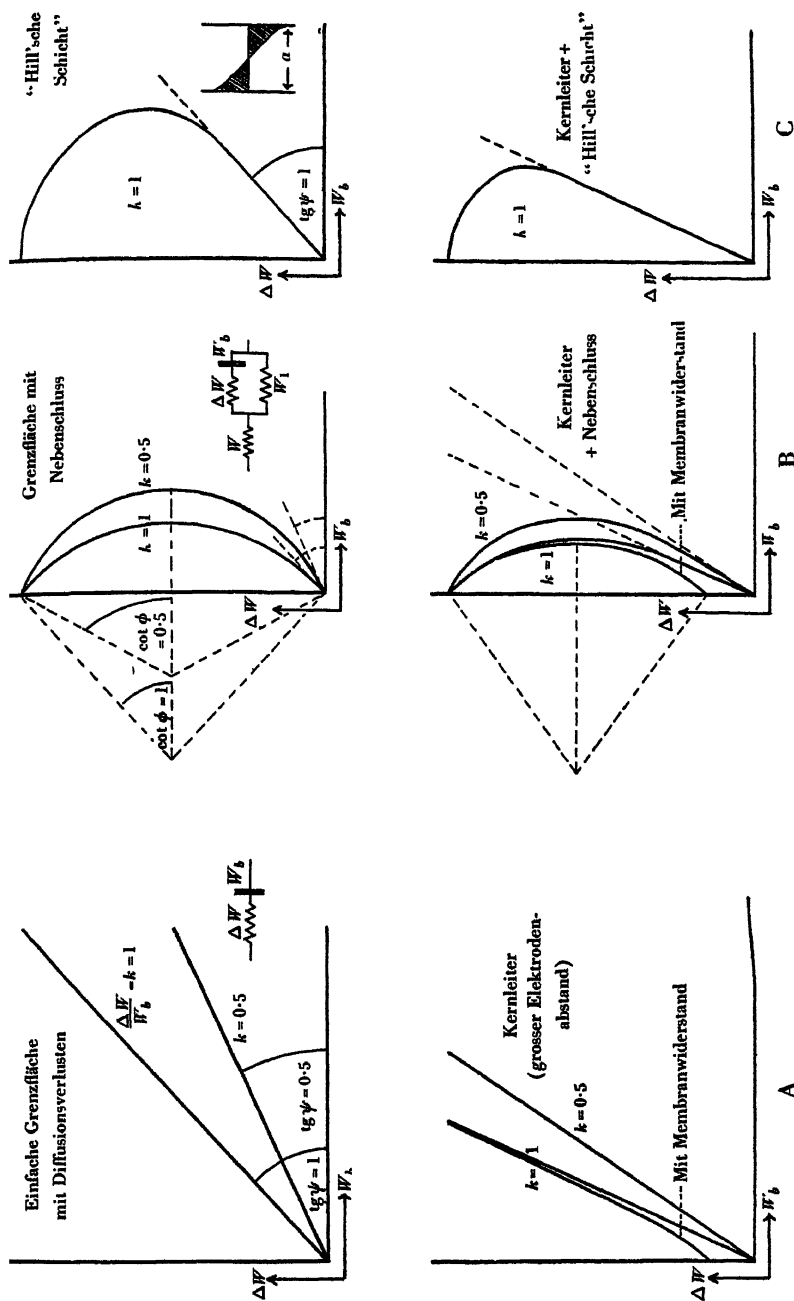


Fig. 4. Die theoretischen Beziehungen zwischen Wirk- und Blindwiderstand polarisierbarer Gebilde bei verschiedenen Frequenzen unter verschiedenen Bedingungen. *Abzisse:* Blindwiderstand W_b . *Ordinate:* polarisatorische Wirkwiderstandsvermehrung ΔW . Obere Reihe: A; Einfache Grenzfläche mit Diffusionsverlusten für einen Verlustwinkel $\text{tg } \psi = 1$ (reine Diffusions-Kapazität), bzw. $\text{tg } \psi = 0.5$ (teilweise Doppelschichtkapazität). B: Dieselbe Grenzfläche mit unpolarisierbarem Nebenschluss. Die Kurve ist ein Kreisabschnitt (Radius r). $\cot \phi = \text{tg } \psi$ ergibt den Verlustwinkel der Grenzflächen *ohne* Nebenschluss, $2r \sin \phi =$ Nebenschluss. C: Dieselbe Grenzfläche ($\text{tg } \psi = 1$) mit Verlusten durch eine "Hill'sche Schicht"; Konzentrationsänderung Δc im Abstand $a/2$ von der Grenzfläche $= 0$. Untere Reihe: Dieselbe Grenzfläche unter entsprechenden Bedingungen, wie oben, aber *im Kernleiter* bei "unendlich grossem Elektrodenabstand". Dieser Abstand ist praktisch beim Froschschladius bei 3–4 mm. langer Strecke schon für Frequenzen bis zu 30 Hertz herab erreicht.

quenzen. Die Genauigkeit der vorliegenden Messungen reicht für endgültige Schlussfolgerungen vorläufig noch nicht aus.

Berechnung der Stromstärken für konstante Spannung V und konstante gespeicherte Elektrizitätsmenge Q an den Grenzflächen

Man kann jedoch trotzdem für die verschiedenen Möglichkeiten aus den direkt gemessenen Widerstandswerten die polarisatorischen Gegenspannungen an den

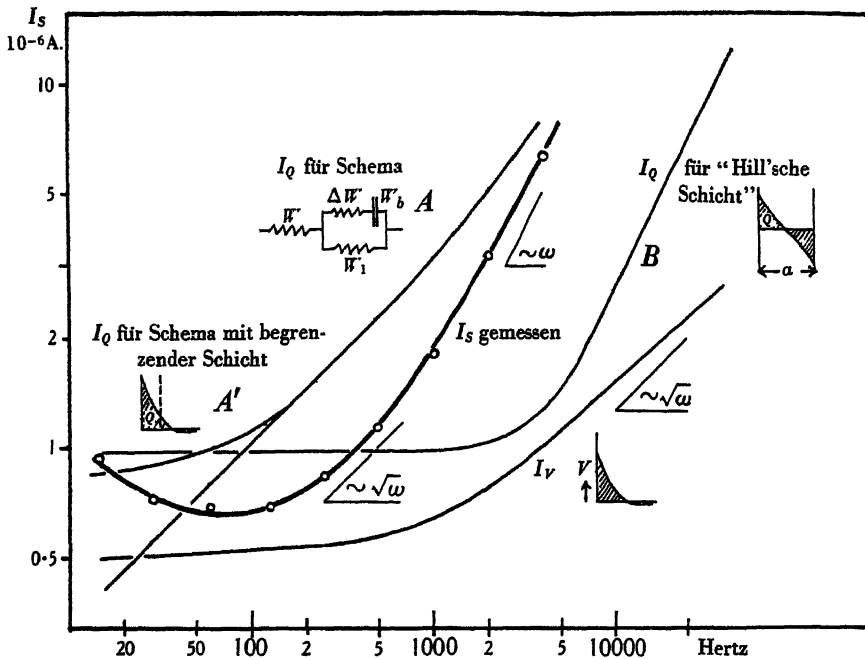


Fig. 5. Aus den gemessenen Werten des Wirk- und Blindwiderstandes eines Nerven berechnete Stromstärken, die an den polarisierbaren Grenzflächen unter gewissen Annahmen bei verschiedenen Frequenzen bestimmte Veränderungen bewirken. I_S = tatsächliche gemessene Schwellenstromstärke. I_V = Stromstärke, die an den gemessenen Grenzflächen eine bestimmte polarisatorische Gegenspannung bewirken würde. I_Q = Stromstärken, die die Speicherung einer bestimmten Elektrizitätsmenge (Gesamtkonzentrationsänderung) an den gemessenen Grenzflächen bewirken würden: A, wenn Verluste in einem Nebenschluss zusammengefasst würden; A', wenn nur ein Bruchteil dieser Gesamtkonzentrationsänderung innerhalb einer bestimmten Schicht an den Grenzflächen wirksam wird; B, wenn Verluste durch den geringen Abstand a der polarisierbaren Grenzflächen (Hill'sche Schicht) bedingt sind. Abszisse: Logarithmus der Frequenz (Hertz). Ordinate: Logarithmus der Stromstärke, für I_S in 10^{-6} A., für die anderen Kurven $\log I +$ willkürliche Konstante.

Grenzflächen durch Rechnung bestimmen und kann auch die Elektrizitätsmengen, die an den Grenzflächen bei Strömen verschiedener Frequenzen gespeichert werden berechnen, um so wenigstens den Versuch zu machen, die Bedeutung dieser Größen für die Reizwirkung der Ströme klarzustellen. In Fig. 5 ist in Kurve I_S für einen Froschischiadicus die bei verschiedenen Frequenzen tatsächlich gemessene Schwellenstromstärke dargestellt. Es handelt sich um den gleichen Nerven wie in Fig. 2.

Die Kurve I_V gibt die Stromstärke an, die bei verschiedenen Frequenzen an den

gemessenen Grenzflächen des Nerven eine bestimmte polarisatorische *Gegenspannung* V bewirken würde. Die Kurve steigt bei höheren Frequenzen, bei denen die Verluste durch Nebenschlüsse oder eine Hill'sche Schicht zurücktreten, proportional der Wurzel aus der Frequenz an, wie es die Theorie von Nernst verlangt, und nähert sich bei niedrigen Frequenzen relativ langsam einem konstanten Wert. Die Berücksichtigung der Verluste durch die Kernleiterstruktur würde die I_f -Kurve bei höheren Frequenzen unter Umständen noch weniger steil ansteigen lassen.

Berechnet man für ein Schema, dass die zusätzlichen Verluste in einem unpolarisierbaren Nebenschluss zusammenfasst, die Stromstärke I_0 , die an den polarisierbaren Grenzflächen zur Speicherung einer bestimmten *Elektrizitätsmenge* Q führt, so ergibt sich, dass diese Stromstärke bei hohen Frequenzen, bei denen die Verluste gering sind, erwartungsgemäss der Frequenz proportional ist, bei niedrigeren Frequenzen etwa proportional der Wurzel abnehmen muss (Fig. 5 A).

Wenn man berücksichtigt, dass diese Elektrizitätsmenge in Form von Konzentrationsänderungen gespeichert werden, die sich mit abnehmender Frequenz über immer grössere Räume erstrecken, so liegt es nahe anzunehmen, dass für eine physiologische Wirkung nur Veränderungen in Betracht kommen, die innerhalb einer bestimmten Schicht vor sich gehen. In diesem Fall würde die notwendige Stromstärke bei niedrigen Frequenzen grösser werden, und sich einem konstanten Wert nähern, wie es in Kurve A' der Fig. 5 dargestellt ist. Die Kurve kann in einem grossen Frequenzbereich, sehr ähnlich wie die tatsächlich gefundene Kurve der Schwellenstromstärke verlaufen. Die notwendige wirksame Elektrizitätsmenge beträgt für einen Froschischiadicus beiläufig $1-3 \cdot 10^{-10}$ Coulomb (Lullies, 1929 b). Die Dicke der hypothetischen Schicht müsste $1-2 \mu$ betragen, wenn man Diffusionsgeschwindigkeiten, wie sie die gelösten Salze unter gewöhnlichen Bedingungen zeigen, der Rechnung zu Grunde legt.

Nimmt man schliesslich an, dass die Verluste die den charakteristischen Verlauf der Wirk- und Blindwiderstandskurve bewirken, durch die *enge Nachbarschaft* der polarisierbaren Grenzflächen verursacht sind (Hill), und berechnet für den Abstand, der sich aus dem Verlauf der Widerstandskurven ergibt, die Stromstärke, die bei verschiedenen Frequenzen eine bestimmte Gesamtkonzentrationsänderung ergibt, so erhält man das Bild der Kurve B der Fig. 5. Die Stromstärke müsste wieder bei hohen Frequenzen proportional der Frequenz sein, würde sich dann aber schon sehr bald und relativ rasch einem konstanten Wert nähern. Diejenigen Schichten, die das Verhalten des Wirk- und Blindwiderstandes verständlich machen, können jedenfalls nicht das Verhalten der Schwellenstromstärke bei mittleren und niedrigen Frequenzen erklären. Eine etwas bessere Angleichung an die tatsächlichen Verhältnisse wäre durch Berücksichtigung der Kernleiterverluste zu erzielen.

Diese wenigen Beispiele aus einer grossen Zahl durchgerechneter Versuche zeigen, dass der Zeitfaktor der Polarisation, die wir messen, im Ablauf der entscheidenden Vorgänge sicher eine wichtige Rolle spielt. Er reicht aber allein nicht aus, um die Vorgänge richtig zu beschreiben.

V. DER ZEITFAKTOR BEI NIEDRIGEN FREQUENZEN

Wesentlich ist zunächst die Feststellung, dass, sowohl beim Zugrundelegen der Speicherung einer bestimmten Elektrizitätsmenge, als auch bei Annahme einer bestimmten *Spannung* an den Grenzflächen als auslösendes Moment, der Elektrizitätsbedarf bei niedrigen Frequenzen relativ grösser wird, und zwar nicht weil die messbaren Verluste grösser werden, sondern aus anderen Gründen. Die genaue Betrachtung hat gezeigt, dass es nicht möglich ist, durch irgendwelche Annahmen über die tatsächlich gemessene Polarisierung und ihre Wirkungen das "Hochbiegen" der Kurve der Schwellenstromstärke bei niedrigen Frequenzen zu verstehen. Solche speziellen Annahmen führen, wie bei der Kurve *A'* in Fig. 5, stets nur zu einem Konstantwerden der notwendigen Stromstärke bei niedrigsten Frequenzen.

Um das Wiederzunehmen der Schwellenstromstärke bei niedrigen Frequenzen zu verstehen, bleibt nichts anderes übrig, als eine Eigenschaft des Gewebes, das beeinflusst wird, heranzuziehen, eine Eigenschaft, wie sie schon Nernst als "*Akkommodation*" bezeichnet hat. Es müsste sich um einen Vorgang handeln, der mit einer gewissen Geschwindigkeit der Wirkung der polarisatorischen Veränderung entgegenwirkt. Er könnte beherrscht sein von einer Konstanten, die der Grösse λ , der Zeitkonstanten der Akkommodation in der Theorie von Hill (1936) entspricht.

VI. DER ZEITFAKTOR BEI HÖHEREN FREQUENZEN

Bei höheren Frequenzen stimmt der Verlauf einer für eine konstante an der Grenzfläche gespeicherte Elektrizitätsmenge berechneten *I*-Kurve mit der Kurve der gemessenen Schwellenstromstärke am besten überein. Um jedoch bei *mittleren* Frequenzen ebenfalls Übereinstimmung zu erzielen, müsste auch hier, sowohl beim Zugrundelegen des Nebenschluss-Schemas, wie bei der Annahme von Verlusten durch eine "Hill'sche Schicht", eine Konstante herangezogen werden, die die Polarisationsmessung nicht unmittelbar ergibt. Es ist die Annahme einer Schicht bestimmter Dicke, also die Einführung einer Struktur-Konstanten notwendig, um das Verhalten der Schwellenstromstärke verständlich zu machen. Diese Grösse hinge jedoch zunächst nicht direkt mit der gemessenen Polarisierung zusammen. Ein Grenzflächenabstand, der die Zunahme der notwendigen Schwellen-Elektrizitätsmenge bei mittleren Frequenzen verständlich macht, müsste etwa 3mal so gross sein, wie die Konstante desjenigen Abstandes, der aus dem Verlauf der Widerstandskurven abzuleiten ist.

Wenn man berücksichtigt, dass die Annahme einer bestimmten Elektrizitätsmenge, als Konzentrationsänderung in einem bestimmten *Raum*, als auslösendes Moment, auch sonst gewisse Schwierigkeiten macht, dann erscheint es angebracht, auf die an sich nächstliegende Vorstellung von Nernst zurückzukommen, nach der die *Spannung* an der Grenzfläche das Entscheidende ist, obwohl auch in diesem Falle die Einführung einer zweiten Zeitkonstante nötig ist, die sich zunächst nicht aus der Polarisationsmessung ergibt.

Es wären zwei Möglichkeiten zu erwägen: *Entweder* eine zweite *Gewebskonstante*, etwa die endliche Geschwindigkeit eines sekundären Prozesses, bestimmt das

Verhalten des Nerven bei höheren Frequenzen und bewirkt die stärkere Zunahme der Schwellenstromstärke in diesem Frequenzgebiet, *oder* aber, man muss annehmen, dass die polarisierbaren Grenzflächen, die bei den untersuchten Nerven die Polarisation beherrschen, nicht diejenigen sind, an denen sich die entscheidenden Vorgänge abspielen. Es wäre möglich, dass andere Grenzflächen mit anderen Eigenschaften hinter den Grenzflächen liegen, die wir messen, eine Möglichkeit, die auch Bishop (1928) diskutiert.

Diese Grenzflächen müssten eine relativ grosse Kapazität haben, also wenig polarisierbar sein, sodass sie das Messresultat wenig beeinflussen, und sie müssten wenigstens bei höheren Frequenzen, fast verlustlos speichern, also im Wesentlichen sogenannte Doppelschichtenkapazität besitzen. Diese würde von einer bestimmten Elektrizitätsmenge jeweils zu einer bestimmten *Spannung* aufgeladen werden. Dass Grenzflächen mit solchen Eigenschaften bei höheren Frequenzen das Verhalten des Nerven elektrischen Reizen gegenüber befriedigend zu beschreiben erlauben, ist von Gildemeister (1927, 1928 a, 1929) in gründlichen theoretischen Untersuchungen nachgewiesen. Bei niedrigeren Frequenzen müssten die üblichen Verluste einsetzen, die, wie die I_V -Kurve in Fig. 5 zeigt, zu konstanten Werten der Schwellenstromstärke bei niedrigen Frequenzen führen. Das Vorhandensein derartiger Grenzflächen wäre auch im Hinblick auf Vorstellungen von Rushton (1935) von Interesse, der als den entscheidenden Vorgang bei der Nervenregung die Veränderung der Oberflächenspannung durch die Aufladung von Grenzflächen in Betracht zieht.

Gewisse Abweichungen im Verlauf der gefundenen Widerstandskurven bei hohen Frequenzen lassen in der Tat an diese Möglichkeit denken, ähnlich wie Messungen an der Haut deutlich zwei hintereinanderliegende Schichten von verschiedenem Charakter, solche mit "umkehrbaren" und "unangreifbaren" Grenzflächen, erkennen und von einander abgrenzen liessen (Lullies, 1928). Das Vorhandensein solcher Grenzflächen im Nerven lässt sich jedoch wegen der Kleinheit des Effekts, der gemessen werden müsste, vorläufig nicht beweisen, andererseits auch nicht ausschliessen.

Wenn man die relativ starke Polarisierbarkeit des markhaltigen Nerven auf seine Hüllen, vor allem auf die Markscheiden zurückführt, deren starke Polarisation den Effekt der vermuteten massgebenden Schichten verdeckt, so musste der Versuch gemacht werden, durch Messungen an *marklosen* Nerven eine Entscheidung herbeizuführen. Man durfte erwarten, dass hier die verschleiernenden Schichten fehlen.

VII. POLARISATION UND SCHWELLENSTROMSTÄRKE IM MARKLOSEN NERVEN

Unter diesen Gesichtspunkten sind solche Messungen an verschiedenen marklosen Nerven durchgeführt (Lullies, 1934 a), jedoch auch vorläufig ohne entscheidendes Ergebnis. Die Effekte an diesen hypothetischen Grenzflächen liegen jedenfalls, bei der Veränderlichkeit biologischer Gebilde, an der Grenze des Messbaren, auch wenn die Markscheide fehlt.

Fig. 6 zeigt die Messungsergebnisse an einem Extremitätennerv von *Maja*

squinado. Der *Maja*-Nerv steht in seinem Verhalten dem markhaltigen Froschischiadicus nicht nur physiologisch sondern auch anatomisch noch verhältnismässig am nächsten. Seine dicksten Fasern besitzen noch Scheiden, die sich mit Osmiumsäure intensiv schwärzen.¹ In Bezug auf Polarisation und Erregbarkeit nimmt er ebenfalls eine Art Mittelstellung zwischen den markhaltigen Nerven und solchen marklosen Nerven ein, die aus dünnsten Fasern bestehen und kaum noch derartiges mit Osmiumsäure zu schwärzendes Material in ihren Hüllen enthalten, wie das ebenfalls untersuchte Mantelkonnektiv von *Octopus* und der Fussnerv von *Aplysia*.

Man sieht in dem Diagramm, wie sämtliche Kurven, die der Polarisation und der Schwellenstromstärke gegenüber denen des markhaltigen Froschischiadicus

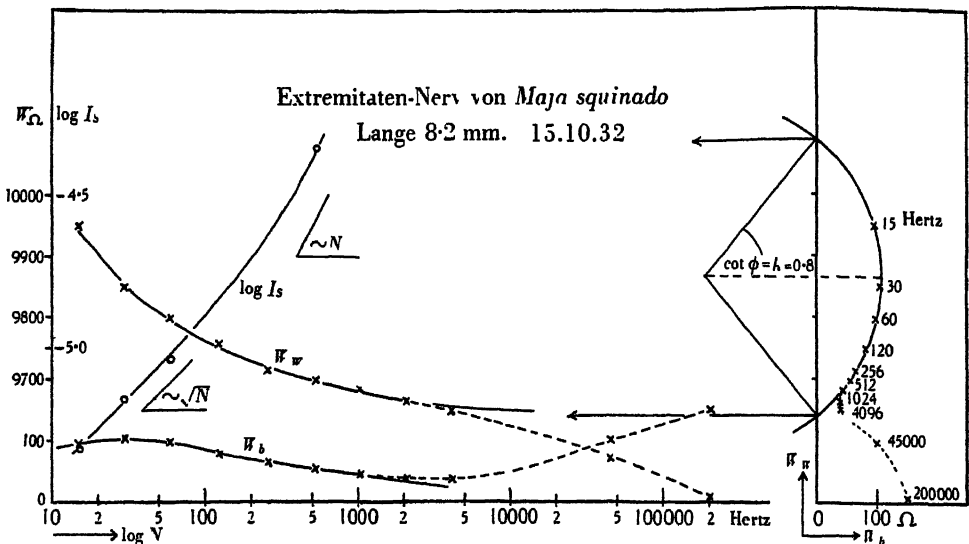


Fig. 6. Frequenzabhängigkeit des Wirkwiderstandes W_w , des Blindwiderstandes W_b und der Schwellenstromstärke I_s für das 8.2 mm. lange Stück eines Extremitätennerven von *Maja squinado*. Rechts ist wie in Fig. 2 der Zusammenhang zwischen Wirk- und Blindwiderstand bei den verschiedenen Frequenzen dargestellt. Die Messungen mit Frequenzen über 2000 Hertz sind unsicher.

nach links zu niedrigen Frequenzen hin verschoben sind, die Widerstandskurven beim *Maja*-Nerven um $1\frac{1}{2}$ –2, bei *Octopus* und *Aplysia* um mehr als 2 Zehnerpotenzen. Im Prinzip verlaufen sie jedoch sehr ähnlich wie beim markhaltigen Nerven.

Um die Ergebnisse ebenso auswerten zu können, wie am markhaltigen Nerven, fehlen Messungen mit entsprechend niedrigen Frequenzen, die bei der Ausführung der Versuche nicht zur Verfügung standen. Ausserdem sind die Messungen der Polarisationsgrössen mit höheren Frequenzen noch mit einer beträchtlichen Unsicherheit behaftet. Der Blindwiderstand und die scheinbare Widerstandsvermehrung betragen hier nur einige Tausendstel des Gesamtwiderstandes. Die

¹ Sie als "Markscheiden" zu bezeichnen scheint nach den Untersuchungen von Young (1935), unzweckmässig zu sein. Man sollte nicht von markhaltigen und marklosen, sondern von markreichen und markarmen Fasern sprechen.

Messung der Frequenzabhängigkeit dieser Grössen wird entsprechend schwierig und ungenau. In diesem Bereich sind die Widerstandskurven im Beispiel der Fig. 6 durch unterbrochene Linien dargestellt. Die möglichst vorsichtige Verwertung der Resultate scheint tatsächlich eine bessere Konstanz von V , der für die verschiedenen Schwellenstromstärken berechneten *Spannung*, an den Grenzflächen zu ergeben. Die aus einer Reihe von Messungen abgeleitete mittlere Spannung an den Grenzflächen errechnete sich für

<i>Maja</i> (Extremitätennerv)	zu 3·5–6 mV.
<i>Octopus</i> (Mantelkonnektiv)	zu 2·5–5 mV.
<i>Aplysia</i> (Fussnerv)	zu 5–8 mV.

Im Übrigen ist es bemerkenswert, dass sich bei den verschiedenen Nerven das Minimum der Schwellenstromstärke in ähnlicher Weise nach niedrigen Frequenzen hin verschiebt, wie das Maximum des Blindwiderstandes, wenn auch nicht in gleichem Masse, sodass man versucht wäre, nach einer Beziehung zwischen beiden Tatsachen zu suchen. Die Lage des Minimums wird jedenfalls mitbestimmt durch die Zeitkonstante der Polarisation.

Diese Betrachtung, bei der man die entscheidenden Vorgänge an Grenzflächen verlegt, denen man die nötigen Eigenschaften zuschreibt, die uns aber nicht, oder nur schwer zugänglich sind, ist bis zur weiteren Klärung nicht sehr befriedigend. Es erhebt sich die Frage, ob es nicht zweckmässig oder gar notwendig ist, den Versuch, alles aus dem physikalischen Vorgang der Polarisation heraus zu verstehen, aufzugeben, und die andere oben angedeutete Möglichkeit zu erwägen. Es könnte bei höheren Frequenzen die Reaktionsweise des Gewebes (ähnlich wie bei niedrigen Frequenzen die "Akkommodation") das zeitliche Verhalten des Nerven entscheidend beeinflussen. Wenn der Vorgang, der sich an die elektrische Veränderung anschliesst, mit einer endlichen Geschwindigkeit abläuft, bis ein Instabilitätspunkt erreicht ist und die Erregung eintritt, so müsste allein auf Grund dieser Tatsache die Schwellenstromstärke oberhalb gewisser Frequenzen mehr und mehr proportional der Frequenz wachsen.

VIII. POLARISATION UND SCHWELLENSTROMSTÄRKE UNTER VERÄNDERTEN AUSSENBEDINGUNGEN

Welchen Anteil bei dem Verhalten des Nerven die Polarisation, und welchen Einfluss die Geschwindigkeit von "Gewebsprozessen" hat, kann im Prinzip durch Messungen der beschriebenen Art unter verschiedenen Bedingungen entschieden werden. Darin liegt die Bedeutung der hier erörterten Methoden. Es ist notwendig, Messungen der Polarisation und der Schwellenstromstärke an markhaltigen und marklosen Nerven, möglichst an solchen, die aus einheitlichen Fasergattungen zusammengesetzt sind, anzustellen. Dabei müssen die Aussenbedingungen von denen man vermuten darf, dass sie Polarisation und Gewebsprozesse in verschiedenem Masse, vielleicht sogar in entgegengesetztem Sinne beeinflussen, variiert werden. In Frage kommen vor allem Änderungen der Streckenlänge, der Temperatur und der Elektrolyt-Konzentration, sowie die Anwendung von Narcoticis und

Giften. Die systematische Untersuchung dieser Fragen befindet sich erst in den Anfängen.

Bei Änderungen des *Calcium-Gehaltes* wird die Polarisierung des Froschischiadicus in eigenartiger, und zunächst überraschender Weise verändert:

Erhöhung der Ca-Konzentration in der umgebenden Ringerlösung auf das 2- und mehrfache des normalen Gehaltes führt zu einer *Verminderung* der Polarisierung. Die Wirkwiderstandsvermehrung und der Blindwiderstand können auf die Hälfte, oder sogar auf ein Drittel sinken. Das Calcium, dessen "abdichtende" und polarisationssteigernde Wirkung bekannt ist, muss hier eine Wirkung an bestimmten Strukturen entfalten, die die Verluste, besonders bei niedrigen Frequenzen, vergrößert. Man könnte mit Labes an eine "Schrumpfung", d. h. Verschmälerung einer "Hill'schen Schicht" denken, oder überhaupt an eine Verkleinerung der polarisierbaren Anteile gegenüber den unpolarisierbaren Bestandteilen des Nerven, die als Nebenschlüsse wirken.

Calcium-Entziehung wirkt für gewöhnlich ebenfalls polarisationsvermindernd, im Gegensatz zu einer Messungsreihe von Labes (1932 c) der unter dem Einfluss von Na-Oxalat eine Zunahme des Wirk- und Blindwiderstandes fand. Der Gesamt-Froschischiadicus scheint hiernach in der Nähe des normalen Calcium-Gehaltes der Ringer'schen Lösung das Maximum seiner Polarisierbarkeit aufzuweisen. Nur durch ganz geringe Änderungen im Ca-Gehalt der Ringerlösung kann mitunter die Polarisierung noch merklich gesteigert werden. Die beobachteten Änderungen der Polarisierung müssen das Ergebnis einer Ca-Wirkung auf zwei verschiedene Strukturbestandteile des Nerven sein.

Die *Schwellenstromstärke* nimmt mit Zunahme der Ca-Konzentration regelmässig zu. Gleichzeitig wird die I_s -Kurve flacher, das Minimum verschiebt sich nach niedrigen Frequenzen. Die Kurve der Schwellenstromstärke ändert sich also zum Teil unabhängig von der Änderung der Polarisierung, wodurch man jedenfalls, mit Solandt zwei von einander unabhängige, durch Calcium verschieden beeinflusste Prozesse abgrenzen kann.

Vermehrung des *Kalium-Gehaltes* setzt die Polarisierung herab, vermindert jedoch gleichzeitig die Schwellenstromstärke. Das Frequenzminimum der Schwellenstromstärke verschiebt sich nach höheren Frequenzen (nach rechts) und die Kurve der Schwellenstromstärke steigt bei höheren Frequenzen steiler an, als unter normalen Verhältnissen.

Ähnlich beobachtet man bei der *Narkose* Veränderungen der Reizwirkung ohne zunächst ersichtlichen direkten Zusammenhang mit den Änderungen der Polarisierung, obwohl auch solche Änderungen vorhanden sind und gemessen werden können.

IX. SCHLUSSBETRACHTUNG

Eine Formulierung der Vorgänge, wie sie sich aus solchen Messungen als wahrscheinlich ergibt, soll an dieser Stelle noch nicht versucht werden. Hill hat kürzlich (1936) die zeitlichen Verhältnisse bei der Reizung des Nerven durch den elektrischen Strom so dargestellt, dass seine Entwicklungen ihre Gültigkeit behalten,

ganz unabhängig davon, welche physikalische Bedeutung man seinen Zeitkonstanten k und λ , der der "excitatory disturbance" und der "accommodation" beilegt. Wenn man aber einmal den notwendigen Schritt tut, nach der Bedeutung dieser Konstanten zu fragen, so ist es unbedingt nötig zunächst den Zeitfaktor der Polarisation mit in Rechnung zu stellen. Er muss, wenn er k nicht völlig ersetzen kann, sowohl bei k , als auch bei λ zur Geltung kommen. Die teilweise recht unfruchtbare Diskussion über die "Chronaxie" und ihre Bedeutung, beruht offenbar darauf, dass auch in dieser Konstanten, die Zeitkonstante der Polarisation, nicht nur des Gewebes, sondern oft auch von Gebilden, die gar nichts mit dem untersuchten Gewebe zu tun haben, enthalten ist.

Einen aussichtsreichen Weg den Anteil der Polarisation zu bestimmen und damit den eigentlichen Konstanten des Gewebes und der Vorgänge selbst näher zu kommen, bieten die Messungen mit Wechselströmen, von denen hier die Rede war.

X. ZUSAMMENFASSUNG

Die Bedeutung der Polarisation für das Verhalten des Nerven bei der elektrischen Reizung kann nur geklärt werden durch Versuche, bei denen Polarisations- und Reizwirkung eines Stromes gleichzeitig direkt gemessen werden.

In dieser Absicht sind mit sinusförmigen Wechselströmen über einen grossen Frequenzbereich Wirk- und Blindwiderstand verschiedener Nerven unter verschiedenen Bedingungen gemessen. Gleichzeitig wird die Abhängigkeit der Schwellenstromstärke von der Frequenz bestimmt. Unter gewissen Voraussetzungen kann aus den Widerstandsmessungen der Betrag und der Verlauf der Konzentrationsänderung (oder der Ladungsänderung), die an den polarisierbaren Grenzflächen bewirkt wird, berechnet werden. Zur Ergänzung werden Messungen mit rechteckigen Stromstössen und Kondensatorentladungen herangezogen.

Es ergibt sich für den N. ischiadicus des Frosches dass das Verhalten der Schwellenstromstärke bei *niedrigen* Frequenzen nur verständlich wird durch Annahme eines "Gewebsprozesses", der wie die "Akkommodation" nach Nernst und Hill den Elektrizitätsbedarf bei niedrigen Frequenzen steigert, ohne dass die Verluste an den polarisierbaren Grenzflächen grösser werden.

Bei Frequenzen oberhalb 200 Hertz geht die Reizwirkung der an den Grenzflächen gespeicherten Elektrizitätsmenge parallel. Die Spannung, die diese Elektrizitätsmenge an den *gemessenen* Grenzflächen bewirkt, ist jedoch bei verschiedenen Frequenzen durchaus nicht konstant.

Wenn die *Spannung* an den Grenzflächen entscheidend sein soll (Nernst), die an sich befriedigendere Annahme, dann können die Grenzflächen, deren Polarisation in erster Linie gemessen wird, nicht diejenigen sein, an denen sich die entscheidenden Prozesse abspielen. Es müssten andere Grenzflächen dahinterliegen, die bei höheren Frequenzen verlustlos speichern.

In der Annahme, dass an marklosen Nerven die entscheidenden Grenzflächen frei liegen und der Messung besser zugänglich sind, wurden entsprechend Mes-

sungen an Nerven von *Maja*, *Octopus* und *Aplysia* angestellt. Gegenüber dem Froschischiadicus sind alle Widerstands- und Schwellenwerte um $1\frac{1}{2}$ bis $2\frac{1}{2}$ Zehnerpotenzen nach niedrigen Frequenzen hin verschoben. Die Wirkung des Stromes scheint in der Tat besser der berechneten *Spannung* an den Grenzflächen parallel zu gehen. Diese Spannung würde 3–8 mV. betragen. Zu einer endgültigen Entscheidung reicht jedoch das vorliegende Material noch nicht aus.

Auch die Annahme eines Gewebsprozesses endlicher Geschwindigkeit, den die elektrische Veränderung auslöst, könnte befriedigende Übereinstimmung der für konstante Spannung an den Grenzflächen theoretisch abgeleiteten und der tatsächlich gefundenen Frequenzkurven der Schwellenstromstärke bei höheren Frequenzen ergeben. Um zu entscheiden, welchen Einfluss die *Polarisation* auf das Verhalten der Schwellenstromstärke bei verschiedenen Frequenzen hat, und was auf Rechnung von "*Gewebsprozessen*", zu setzen ist, müssen Messungen unter veränderten Aussenbedingungen (Streckenlänge, Temperatur, Elektrolyt-Konzentration, Narcotica, Gifte) angestellt werden. Orientierende Versuche zeigen, dass *Vermehrung* des *Calcium*-Gehaltes im Nerven, ebenso wie Calcium-Verminderung gegenüber der Norm zu einer *Abnahme* der gemessenen Gesamt-Polarisation führt. Das Calcium scheint auf zwei verschiedene Strukturen zu wirken.

Demgegenüber wird die Frequenzkurve der *Schwellenstromstärke* von Calcium einsinnig beeinflusst. Die Schwellenstromstärke wird durch Calciumvermehrung stets erhöht, für hohe Frequenzen relativ stärker, als für niedrige, das Minimum der Frequenzkurve meist nach niedrigen Frequenzen hin verschoben.

Vermehrung des *Kalium*-Gehaltes führt zu einer Abnahme der Polarisation; das Minimum der Schwellenstromstärke wird nach höheren Frequenzen verlagert.

Diese Ergebnisse bestätigen vorläufig nur den Schluss, dass bei der Reizung des Nerven mit niedrigen Frequenzen ein Gewebsprozess abzugrenzen ist von Faktoren, die die Vorgänge bei höheren Frequenzen beherrschen. Es ist aber anzunehmen, dass die Auswertung eines grösseren Materials in der angedeuteten Richtung genauer angeben lassen wird, in welcher Weise und zu welchem Betrage die Wirkung der Polarisation und der Einfluss sekundärer Prozesse im Gewebe in dem zeitlichen Ablauf der Vorgänge bei der elektrischen Reizung zum Ausdruck kommt.

XI. SUMMARY

The importance of polarization in the behaviour of electrically stimulated nerves can only be elucidated by experiments in which the effects of both polarization and stimulus are measured at the same time.

With this in mind, both the resistance and the reactance of different nerves have been measured, sinusoidal alternating current covering a wide range of frequencies being used for stimulation. At the same time the dependence of current threshold on frequency has been investigated. Under certain conditions both the magnitude and the course of change of concentration (or of the change of charge) at the polarizable interfaces can be calculated from the resistance measurements. Supplementary measurements have been made with rectangular current impulses and condenser discharges.

The result, in the case of the sciatic nerve of the frog, is that the behaviour of the current threshold at low frequencies can only be explained intelligibly by the assumption

of a certain process taking place in the tissue, which, like the "accommodation" of Nernst and of Hill, raises the electrical requirements at low frequencies, although there is no increased loss at the polarizable interfaces.

At frequencies about 200 cycles the effect of the stimulus is parallel to the quantity of electricity stored at the interface. However, the potential arising from this amount of electricity at the interface which is being studied is not at all constant at different frequencies.

If the potential at the interface be supposed to be the decisive factor (Nernst), which is in fact the more satisfactory assumption, then the interfaces whose polarization is mainly being measured cannot be those at which the important processes take place. Other interfaces must lie behind them, storing without loss at higher frequencies.

On the assumption that in non-medullated nerves the important interfaces are exposed and more easily accessible for measurement, corresponding measurements were carried out on nerves of *Maja*, *Octopus* and *Aplysia*. Compared with the sciatic nerve of the frog, all resistances and threshold values are shifted towards the lower frequencies by $10^{1.5}$ to $10^{2.5}$. In fact, the effect of the current appears to correspond better with the calculated potential at the interfaces. This potential would be 3–8 mV. The available data, however, are insufficient for final conclusions.

The assumption of a process with a finite velocity, taking place in the tissue and caused by the electrical change, would give a satisfactory agreement between the potential theoretically deduced for constant potential at the interface and the experimentally established frequency relations to the current threshold at higher frequencies. In order to decide what is the influence of polarization on the behaviour of the current threshold at different frequencies, and what is to be attributed to processes taking place in the tissues, measurements under varied external conditions (length, temperature, electrolyte concentration, narcotics, poisons) will have to be carried out. Preliminary experiments show that both increase in the calcium content of the nerve and calcium decrease lead to a decrease in the total amount of polarization as compared with the normal. Calcium appears to act upon two different structures.

The frequency relation of the current threshold, on the other hand, is affected by calcium in one way only. The current threshold is always raised by an increase in calcium, more so at high than at low frequencies, the minimum of the frequency curve being shifted towards the lower frequencies.

Increase in potassium content leads to a decrease in polarization, the minimum of the current threshold being shifted towards the higher frequencies.

Up to the present these results merely support the conclusion that, on low frequency stimulation of the nerve, a process taking place in the tissue can be separated from the factors that govern the processes at higher frequencies. It is probable, however, that further investigations will enable us to state more accurately how and to what extent the effect of polarization and the influence of secondary processes in the tissue are expressed in terms of the time relations of the processes caused by electrical stimulation.

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NACHTRAG

Hill, A. V., Katz, B. and Solandt, D. Y. (1936, *Proc. roy. Soc.*, B, **121**, 74) beschäftigten sich in einer kürzlich erschienenen Arbeit ausführlicher mit der Reizwirkung sinusförmiger Wechselströme auf den Froschnerven, insbesondere auch mit dem Einfluss des Elektrodenabstandes, der Temperatur und des Calciums auf die "Optimalfrequenz". Sie finden unter anderem, dass Calcium die Optimalfrequenz nach *höheren* Frequenzen hin verschiebt, als Ausdruck einer Beschleunigung der "Akkommodation". Wenn in unseren Messungen umgekehrt in der Regel bei Ca-Vermehrung eine Verschiebung der Optimalfrequenz nach *niedrigen* Frequenzen zu gefunden wird, so gibt das Veranlassung, auf einen wesentlichen Punkt bei der Wahl der Versuchsbedingungen hinzuweisen: Mit dem *Aktionsstrom* als Indikator muss die Frequenzkurve der Schwellenstromstärke eines Froschischiadicus stets flacher ausfallen, die Optimalfrequenz muss niedriger liegen, als bei Messung bei denen die *Muskelsuckung* als Indikator für den Reizerfolg dient, und auch diese Kurve wird nicht mit der Frequenzkurve einer Einzelfaser übereinstimmen. Man erhält stets nur die "einhüllende" Kurve derjenigen Gruppe von Nervenfasern, die mit der betreffenden Methodik erfasst wird, und diese "Einhüllende" wird bei äusseren Einwirkungen, die die Nervenfasern verschiedener Eigenschaften verschieden beeinflussen, sehr wohl eine andere Formänderung erfahren können, als die Frequenzkurven der Einzelfasern. Hierauf wird bei weiteren Untersuchungen zu achten sein.

BIOCHEMISTRY OF THE LOWER FUNGI

By J. H. BIRKINSHAW

(Division of Biochemistry, London School of Hygiene and Tropical Medicine,
University of London)

(Received 15 May 1936)

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I. INTRODUCTION

ALTHOUGH the taxonomic literature relating to the lower fungi, commonly known as moulds, runs through two centuries of descriptive botany, the study of the biochemistry of these organisms is of comparatively recent date. Prior to the last decade of the nineteenth century the impression seems to have prevailed that the chief products of moulds, apart from the actual constituents of the cell substance, were carbon dioxide and water. The classical researches of Wehmer, begun in 1891, showed that moulds could initiate and maintain a true fermentation process, with the formation from sugar, of large amounts of organic substances such as oxalic and citric acids, which were well known as products of higher plants. These researches gave a great impetus to the study of the biochemistry of moulds both by industrial and by purely academic interests.

To the investigator interested in vegetable physiology moulds offer the advantages of rapid growth and ease of culture on synthetic media. On the other hand, any given strain may be found to vary in biochemical characteristics with continued

cultivation under laboratory conditions, but this disadvantage may, perhaps, be obviated with increasing knowledge of the nutritional requirements.

When standard cultural conditions are adopted, both general and specific biochemical characteristics of moulds are frequently found to correspond in a remarkable manner with the taxonomic features; thus the study of the biochemistry of these organisms may assist in the task of classification, which is frequently extremely difficult when morphological features alone are considered. An example of this is to be found in the study of the carbon balance sheets as determined by Birkinshaw *et al.* (1931, III) for species of *Aspergillus*. In this genus the arrangement of the different species according to qualitative and quantitative aspects of the type of products formed follows closely the classification based on morphological characteristics as used by Thom & Church (1926).

In this review of the biochemistry of moulds it is our intention to trace the trend of the more recent developments. Although frequent reference is made to the earlier investigations in order to obtain a correct perspective, only the more outstanding of these early papers are cited. This treatment is justified apart from space considerations, since the value of the work of some of the pioneers in this field is seriously impaired by their use of cultures, the purity of which is open to question, and by their use of inadequately described or wrongly named organisms whose identity cannot now be established.

II. INORGANIC CONSTITUENTS OF THE MEDIUM

The classical researches of Raulin (1869) led him to put forward a fairly complex medium for growth of species of *Aspergillus*. This had the following composition: water 1500 ml., sucrose 70 g., tartaric acid 4 g., NH_4NO_3 4 g., ammonium phosphate 0.6 g., MgCO_3 0.4 g., K_2CO_3 0.6 g., $(\text{NH}_4)_2\text{SO}_4$ 0.25 g., $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ 0.07 g., $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 0.07 g., K_2SiO_3 0.07 g. Raulin held the view that none of the mineral constituents of his formula could be omitted if optimal growth was to be obtained.

Naegeli (1882) regarded as indispensable for moulds the elements (*a*) sulphur, (*b*) phosphorus, (*c*) potassium, which could, however, be replaced by rubidium or caesium, and (*d*) calcium, replaceable by magnesium, barium or strontium. Benecke (1894) and Molisch (1894) as a result of careful experiments concluded that magnesium was indispensable for all moulds examined and could be replaced by no other element, but that calcium could be omitted without any ill effect. The potassium and phosphorus requirement of *Aspergillus niger* have frequently been used as the basis of a method for determining the available potassium and phosphate of soils. Disregarding the rarer elements we might therefore arrive at the generalization that sulphur, phosphorus, potassium, and magnesium are indispensable, but this statement may require modification in the case of magnesium. Rossi & Scandellari (1932) concluded that magnesium was not essential for *Aspergillus niger*, although it had a favourable influence on growth.

Stoess (1932) claimed that calcium had a favourable effect in some cases, although its absolute necessity could not always be demonstrated. He found that

fungi which react favourably to calcium are those which are sensitive to injury by small doses of magnesium, and explained the corrective action of calcium on magnesium injury as being due to colloidal phenomena. Strontium can partly replace calcium in this respect.

The position of the so-called heavy metals, represented in Raulin's formula by iron and zinc, has aroused much controversy. Currie (1917), following Wehmer, and basing his opinion both on his own work and on that of other observers such as Naegeli (1880), Molisch (1894), and Benecke (1895), decided that the medium of Raulin was unnecessarily complicated. He developed an agar medium containing as the only inorganic salts ammonium phosphate, potassium chloride, and magnesium sulphate. This medium was used for maintaining about sixty stock cultures of *Aspergillus* and *Penicillium*. He records that nearly every culture grew more luxuriantly than on many of the more complex media described in the botanical literature.

Meanwhile evidence has been steadily accumulating to show that traces of heavy metals are essential constituents of the medium and are not to be regarded merely as stimulants. Molisch (1892) was the first to recognize that the traces of metals present in the so-called pure chemicals as usually employed for making up media were sufficient to vitiate many of the earlier experiments, and he eliminated iron as far as possible by subjecting his reagents to rigorous purification. Steinberg (1919) developed a new method for purification of the nutrient medium from traces of heavy metals. This consists in treating the nutrient solution with calcium carbonate to increase the alkalinity and to furnish a precipitate to adsorb traces of heavy metal precipitates simultaneously formed. The medium is then autoclaved at one atmosphere and decanted or filtered. Bortels (1927) used absorbent carbon after making the medium alkaline to remove traces of heavy metals, and Roberg (1928) showed that alkalinity alone is sufficient to precipitate most of the traces of heavy metal impurities in the nutrient solution. Both these authors conclude that iron, zinc, and copper are essential elements for the growth of *Aspergillus niger*. In later papers Steinberg (1935 *a, b*) presents evidence for the essential nature of manganese in addition to iron, zinc and copper. He finds that the apparent optima for growth of *A. niger* are: Fe 0.20, Zn 0.14, Cu 0.06, Mn 0.03 mg. per litre. Even 0.001 mg. of zinc is able to bring about an increase of 92.5 per cent in the yield, whilst absence of one of the elements may reduce the yield by 60–98 per cent. He further makes the point that the high dilution may be illusory, since absorption by the cell may result in the concentration of these elements within the protoplast. If any of the four metals are omitted sporulation is inhibited. In the absence of copper, deposition of pigment on the spore walls is interfered with and the spores are brown, yellow, or white, apparently according to the extent of the deficiency. The presence of both iron and zinc seems to be necessary for the acid metabolism, whereas addition of manganese and perhaps copper seems unnecessary. Manganese particularly does not seem to be required for acid formation, and it is surmised that it bears some relation to the processes whereby the acids are decomposed.

The effects of zinc on *A. niger* are confirmed by Porges (1932 *a*). He found that 0.01 per cent of zinc sulphate in a 10 per cent sugar solution tended to retard spore

formation and to increase vegetative growth. There was a greater utilization of sugar and a larger yield of citric acid whilst the ether- and alcohol-soluble constituents and hemicelluloses were increased. The lignin content on the other hand was depressed. Angeletti & Merlo (1934) note that iron has a specific action on the gluconic acid fermentation of *Penicillium luteum purpurogenum*. With small amounts of iron (0.01145 g. per litre) as ferric chloride in a medium containing 20 per cent of glucose the yield of gluconic acid is at first retarded but finally (in 20 days) increased by about 11.5 per cent. Quilico & di Capua (1932) found that the effect of iron on the citric acid fermentation varied with the strain of *Aspergillus niger* employed, the yield of acid being in one case depressed and in another increased with increasing amounts of iron. Variation and particularly restriction in the amounts of the essential elements phosphorus, sulphur and nitrogen causes profound modifications in the acid metabolism of *A. niger*, particularly in the relative amounts of products, as will be shown later with reference to acid production.

Aluminium in small amounts is said to have no action on the sugar metabolism of "*Penicillium glaucum*" (Manceau, 1931), and Lévy (1932) found that whilst 0.005–200 mg. per litre had no effect on the development of *Aspergillus niger*, 350 mg. completely inhibited growth. Most of the heavy metals show a similar inhibitory effect when present in more than traces.

III. NITROGEN METABOLISM

The question as to whether moulds can fix atmospheric nitrogen has been much discussed. Since those workers who claim to have obtained positive results depend for the most part on nitrogen differences of a few mg. or even fractions of a mg., it seems open to doubt as to whether their results are significant. One of the most recent workers on the subject (Roberg, 1932) could obtain no evidence of assimilation of molecular nitrogen by various species of *Aspergillus*, and until definite and incontrovertible evidence to the contrary is forthcoming it appears wiser to accept the view that molecular nitrogen is not available for the lower fungi.

Combined nitrogen in a great variety of forms is utilized by moulds. Most synthetic media supply nitrogen in inorganic form as nitrates or ammonium salts. The nitrogen seems to be equally available in both these forms, although the effect on the reaction of the medium of the removal of an acidic or basic ion with results sometimes detrimental to the culture must not be overlooked. Since moulds are capable of transforming nitrates into ammonia it is only to be expected that either of these forms, and probably any intermediate stage of oxidation, provided it is not definitely toxic, would be available. Among the more recently reported examples of reduction of nitrates and the utilization of compounds in an intermediate state of oxidation may be mentioned the reduction of nitrates with production of ammonia by *A. repens* at low pH (Bach & Desbordes, 1933), the production of hydroxylamine by cultures of *A. niger* in media containing ammonium nitrate (Lemoigne & Desveaux, 1935) and the assimilation of nitrites by various species of moulds (Sakaguchi & Chang, 1934).

Organic nitrogen is readily assimilated in various forms. Ivanov & Osnizkaja (1934) found that *A. niger* on a nitrogen-free medium containing sugar excretes nitrogen into the medium in a form which cannot be utilized, but that when potassium cyanide is added its nitrogen is assimilated, the nitrogen content of the mycelium increasing almost fourfold. Urea is formed from peptone and guanidine by various species of *Penicillium* and *Aspergillus*. Chrzaszcz & Zakomorny (1934) consider that guanidine may be an intermediate in the production of urea from peptone, the precursor of guanidine being arginine. Urea is in itself an excellent source of nitrogen and was found to be the best source for growth and sterol production of *A. fischeri* by Wenck *et al.* (1935) in media free from calcium carbonate, although in presence of calcium carbonate ammonium nitrate was better. Various amino acids, peptones and proteins have been used for the nitrogen source, and indeed it may be said that almost any form of combined nitrogen which occurs in, or results by simple hydrolytic fission of, natural products is available, in addition to many forms of purely synthetic origin.

For nitrogen compounds to exercise their full nutrient value a suitable source of carbon is essential, and the ratio in which the nitrogen and carbon are supplied often has a profound influence on the products formed. The actual source of nitrogen and its quantitative relationship to other nutrients also becomes of great importance when definite fermentation products are desired. Thus to quote only one example, Kinoshita (1927) found that in the production of kojic acid by *A. oryzae* a deficiency in the supply of nutrient nitrogen resulting from the use of cobaltamines as nitrogen sources caused an increased yield of the acid.

IV. SOURCES OF CARBON

Since moulds do not contain chlorophyll they are unable to utilize atmospheric carbon dioxide for tissue synthesis. They must therefore be supplied with organic carbon, which serves not only as a source of cell substance but also supplies their energy requirements.

The sources of available carbon are many and varied, and will depend to some extent on the species, but the observations of Tamiya (1932 *a*) for a particular species may be taken to some extent as representative of the group. Tamiya found that *A. oryzae* was able to utilize fifty-one organic compounds, mostly alcohols and acids, out of a total number of 123 tested for growth and respiration. Of the remainder, eight were available for respiration only and seventeen were without effect. The remaining forty-seven substances were deleterious. Certain groups such as CO and CHO were found to inhibit growth. The organism utilizes many sugars and pyruvic acid, but not alcohols, acids, aldehydes, ketones or ethers for anaerobic fermentation. He concluded that certain pairs of groups, e.g. $\text{CH}_3 \cdot \text{CH}(\text{OH}) \cdot$, $\cdot \text{CH} \cdot \text{C}(\text{OH}) \cdot$, $\text{CH}_2\text{OH} \cdot \text{CH}_2 \cdot$ which he termed the "chief radicals" must be present in a utilizable compound and that they must be joined at least once (in ring or straight chain) to another group termed the "residual radical" such that β degradation does not lead to a fission of the chief radical. Fission of di- and

polysaccharides and of glucosides precedes their utilization by the mould, and the nascent fission products can usually be utilized better than the preformed substance.

Among the more complex sources of available carbon which have recently been studied may be mentioned olive oil, utilized by *A. flavus*, *Penicillium sylvaticum* and a white fungus (Katz-Nelson, 1931), higher paraffins with chains not exceeding $C_{31}H_{70}$ utilized by *Aspergillus versicolor* (Hopkins & Chibnall, 1932), triolein, linseed and walnut oils utilized by species of *Penicillium* (Oeffner, 1931), pentosans and pentoses utilized by *Aspergillus* species (Horowitz-Wlassowa & Novotelnov, 1935), amylopectin used by *A. niger* (Härdtl, 1933), cellulose (Norman, 1931).

The relation of source of carbon to fermentation product is exceedingly complex and cannot be adequately discussed, but will be mentioned as occasion arises when mould products are considered.

V. RESPIRATION AND ENERGY EXCHANGE

Moulds are essentially aerobic organisms, and no case has yet been recorded of a mould growing under strictly anaerobic conditions, although some species can flourish in an atmosphere of comparatively low oxygen tension. Thus Thom & Currie (1913) found that *Penicillium roqueforti* exists in almost pure culture in cheese since it is specially adapted to survive the conditions prevailing in the interior of the cheese. In a comparison of several species they found that *P. roqueforti* was able to sustain a lower oxygen and higher carbon dioxide tension than any other organism examined.

Although moulds on liquid media are usually grown as surface films which have ready access to atmospheric oxygen, the literature records several attempts to carry out mould fermentations with submerged mycelia; in such cases the essential oxygen supply is introduced into the liquid by bubbling or agitation. An example of this is the production of gluconic acid in the special form of apparatus employed by May *et al.* (1934), where the air is supplied under pressure. A case of subsurface fermentation without special air supply has recently been described by Yuill (1936). The organism, *Aspergillus flavus* Link, produces a thick submerged mycelium when grown on a medium containing chalk. Under these conditions the products differ from those obtained with surface growth. Here the mould is dependent on oxygen dissolving in the aqueous medium and diffusing to the mycelium, which is necessarily a slow process. A further example of the influence of decreased aeration on fermentation products is provided in the conversion of glucose to mannitol by a white species of *Aspergillus*, as described by Birkinshaw *et al.* (1931, ix). Here the yield of mannitol was found to be largely dependent on the restriction of the air supply.

The energy balance of growth appears to have first been studied by Terroine & Bonnet (1927). Terroine & Wurmser (1932) found that the energy yield is reduced by half by the increased respiration caused by growth. Tamiya (1932 *b*) states that the respiration quotient is proportionately greater or less than the combustion quotient of the substrate according as to whether the combustion quotient is greater or less than 0.875, which is the value of the combustion quotient of the mould

constituents. The energy resulting from respiration during vital synthesis appears to be mainly utilized for (a) maintenance of enzymic and structural energy; (b) replacement of heat loss in certain stages of synthesis; (c) activation of substrate necessary for the acceleration of the velocity of the reactions during synthesis. The relation of the respiration rate to growth is expressed by Tamiya & Yamaguchi (1933) as an equation of the second degree. Yamagata (1934), although confirming the respiration quotient theory of Tamiya, finds that the value of the combustion quotient is 0.91–0.95, not 0.875. For all sources of carbon the respiration quotient of *A. oryzae* is greater when the nitrogen is derived from potassium nitrate than when it is obtained from ammonium salts. With nitrate substrates the excess of carbon dioxide from nitrate reduction is liberated, and a means of determining this excess in the growing cell is derived. The energy set free in combined oxidation-reduction processes in nitrate reduction is probably lost as heat, so that on such substrates the growing cell must perform additional work in order to reduce nitrates.

In considering the respiration of moulds the action of respiratory poisons is of some interest. Yamamoto (1933) showed that the true respiratory poisons, e.g. potassium cyanide, act firstly on the respiration and secondly on the growth of *A. niger*, thus producing a preliminary increase in utilization of respiratory energy. Other poisons, e.g. phenylurethane, carbon monoxide, sodium fluoride, primarily decrease growth, which is reflected in diminished utilization, whereas iodoacetate acts on both processes, so that energy utilization remains approximately constant.

VI. GROWTH FACTORS

Various substances specifically stimulate or inhibit some of the metabolic processes of moulds. Only organic stimulants and inhibitors will here be considered, since as already stated the so-called stimulants to be found amongst the metals are probably essential elements and as such have already been discussed. Tomkins (1932) found that in presence of acetone, chloroform and ether vapour the latent period of germination of various moulds is the same as in air, but acetaldehyde, hydrocyanic acid and hydrogen sulphide retard germination and check the growth rate. May *et al.* (1932) examined a number of organic compounds for their effect on kojic acid production by *A. flavus*. Only ethylene chlorhydrin (in amounts of 100 mg. per litre) showed a definite stimulation, whilst thiourea, thio-glycollic acid, sodium thiocyanate, chloroacetone and *o*- and *p*-chlorophenol were inhibitory. Various anti-oxidants and substances influencing the respiratory exchange, e.g. methylene blue and 2:4-dinitrophenol, do not affect the growth or composition of *A. niger* according to Bonnet & Jacquot (1934).

Certain alkaloids affect the utilization of carbohydrates by *A. niger*. Thus the utilization of glucose or sucrose on Wehmer's or Raulin's medium was found by Mezzadrolì & Amati (1933) to be enhanced by the presence in solution of 0.05–0.2 per cent of strychnine as nitrate or 0.05–0.3 per cent of quinine as sulphate, but 0.05–0.3 per cent of caffeine (base) had the opposite effect.

A somewhat mysterious substance which promotes the growth of *A. niger* was

observed by Nielsen & Hartelius (1932) to be formed by the interaction of carbohydrates, organic acids or their ammonium salts and filter paper or its ash, especially at high temperatures, as when autoclaved at 135° C. The stimulant was not isolated but apparently contains no nitrogen, is insoluble in ether and is destroyed by hydrogen peroxide. It is most probably a degradation product of the sugar.

Williams & Honn (1932) found that the growth of moulds and certain yeasts was stimulated by certain vitamin-like substances supplied in the form of yeast extract, and describe similar effects obtained with various amino acids which induced freer sporulation. It appears to be definitely established that the vitamins of the B group have a favourable effect on moulds. Thus Solheim *et al.* (1933) found that vitamin B₁ and B₂ increased the fructification of certain fungi, and in the case of *A. niger* and certain *Penicillia* induced yellow pigmentation. Schopfer (1934 *a, b*) states that normal development of *Phycomyces* is impossible in purely synthetic media, but that addition of crystalline preparations of vitamin B₁ or B₂ induces rapid growth, which increases with the supply of nitrogen. The action of vitamin B₁ is optimum at pH 7.0–7.2, is unaffected by heating at 135° C., but is much reduced by autoclaving with alkali, which would naturally lead to destruction of the vitamin. He further finds (1935 *a*) that the vitamin B₁ is actually absorbed by the fungus from the nutrient medium, which gradually loses its ability to activate other media. Further, extracts of *Phycomyces* grown on an active medium can activate other media, a property which immediately becomes manifest as soon as a trace of B₁ is added to the original culture. Schopfer suggests that the organism synthesizes another growth factor in proportion as it is supplied with B₁. The stimulatory action of certain plant extracts, e.g. wheat germ, orchid pollinia, and rice polishings (Schopfer, 1934 *a*) may be due to their vitamin content, but this is more doubtful in the case of fruit juice (orange, tomato), lily or gladiolus bulbs, potato or dahlia tubers (Wilcoxon & McCallan, 1934). Vitamin C and inositol with "bios II" were found by the last-named authors to be ineffective.

Some moulds have the power of elaborating substances which are inhibitory to other micro-organisms. An outstanding example is *Penicillium chrysogenum* Thom, which yields a product which was named "penicillin" by its discoverer, Fleming (1929). This substance, even in high dilution, inhibits the growth of the pyogenic cocci and the diphtheria group of bacilli, but is readily tolerated by many other bacteria, e.g. the coli-typhoid group, the influenza bacillus group and the enterococcus. Reid (1935) showed that an organism (*Staphylococcus aureus*) sensitive to penicillin was not destroyed but merely inhibited. He further showed that light, oxygen, hydrogen, and carbon dioxide prevented the formation of penicillin or destroyed it, although it is relatively stable to heat. Haenseler (1934) recorded that *Rhizoctonia* failed to grow in media previously carrying *Trichoderma*. The toxin produced was destroyed by heating at 80° C. for 10 min., by ageing for 10 days, or by exposure to oxygen for 5 min.

The staling agent produced by *Helminthosporium sativum* was found by Carter (1934) to be stable to sterilization and to migrate to the negative pole in a Bradfield dialyser.

VII. METABOLIC PRODUCTS

The classical researches of Wehmer may be regarded as having initiated the study of the metabolic products of the lower fungi, although a few scattered observations by earlier observers working for the most part with unauthenticated or impure strains are to be found in the literature.

(1) *Products containing not more than six carbon atoms*

Oxalic acid. Earlier observers had noted crystals of calcium oxalate present in the mycelium of many moulds, but Wehmer (1891 *a, b*, 1897) showed that oxalic acid was produced in considerable yield by *Aspergillus niger* on a variety of substrates and recognized it as a definite fermentation product. He was able to increase the yield by addition of calcium carbonate to the medium. Other investigators have confirmed the work of Wehmer and have shown that oxalic acid is produced not only by various species of *Aspergillus* but also by *Penicillia*, e.g. *P. oxalicum* (Currie & Thom, 1915).

Citric acid. The credit of the discovery of citric acid as a mould fermentation product is again due to Wehmer (1893 *a, b*). He introduced the genus *Citromyces* to include fungi producing citric acid from sugar and described two new species, *C. glaber* and *C. Pfefferianus*, having this characteristic. He found that the acid was produced from various sugars and again employed calcium carbonate in the medium to neutralize the acid formed and so to improve the yield. It was not long before many other species of other genera, notably *Aspergillus* and *Penicillium*, were found to produce citric acid in equal or greater yield. The naming of the genus *Citromyces* was therefore somewhat unfortunate, and the necessity for the creation of a separate genus for this group of moulds is disputed by some mycologists. Wehmer himself (1897) reported that citric acid is produced from sucrose by a variety of *P. luteum*, and Currie (1917), in investigating the lag between titratable acidity and oxalic acid production by *Aspergillus niger* on sucrose, discovered that citric acid was also formed. By a correct adjustment of the conditions of fermentation the oxalic acid production could be almost entirely suppressed. The process is now used commercially for the production of citric acid in various countries where the climate is unsuitable for the cultivation of citrus trees.

A number of hypotheses have been advanced to account for the transformation of the straight carbon chain of the sugars into the branched chain present in citric acid, but so far the evidence is insufficient to elucidate the mechanism. Some of these hypotheses can, however, be eliminated. Thus the postulation of acetic acid as one of the intermediate stages has suggested a preliminary breakdown of hexose similar to that encountered in alcoholic fermentation by yeast. Clutterbuck (1936) and Wells *et al.* (1936) point out that such a breakdown plays no part in the formation of citric acid by moulds. For the theory as developed by Bernhauer (1934) to hold, the weight ratio of citric acid to carbon dioxide should not exceed 1.45 : 1, and the weight yield of citric acid from glucose should not exceed 71.1 per cent, but ratios up to 4 : 1 and yields up to 90 per cent were actually obtained by Wells *et al.*

Emde (1935) suggested the formation of quinic acid as an intermediate, but on his hypothesis the maximum yield of citric acid from sucrose would be only 56 per cent and correspondingly less from a hexose. On the other hand, the hypothesis of Raistrick & Clark (1919) is still tenable on a yield basis. In this hypothesis the hexose first affords $\alpha\gamma$ -diketoadipic acid, which is then hydrolysed to acetic and oxalacetic acids. These two acids then combine to form citric acid. Even this mechanism, however, is inadequate to account for the production of citric acid from all the various substrates from which it is obtained unless we assume that in all cases a hexose is first formed by synthesis.

d-Gluconic acid. This acid was first isolated from the products of mould metabolism by Molliard (1922). He showed that it is formed from sucrose by *Aspergillus niger*. Other workers in this field have shown that gluconic acid is produced from various sugars by *A. niger* (Bernhauer, 1924, 1926, 1928), by *Citromyces glaber* and *Penicillium glaucum* (Butkewitsch, 1925), by *Aspergillus niger*, *fuscus* and *cinnamomeus* (Falck & Kapur, 1924), by *Penicillium purpurogenum* var. *rubrisclerotium* (May *et al.* 1927), by *P. chrysogenum* and *Fumago vagans* (Birkenshaw & Raistrick, 1931, xvii), by *Penicillium crustaceum* (L.) Fries (Angeletti, 1932) and by *Aspergillus oryzae* (Sakaguchi, 1932). The evidence available seems to indicate that gluconic acid is produced by a simple oxidation of glucose.

The three acids, oxalic, citric and gluconic acid, have been considered first, since they can be obtained in large yield and are amongst the commonest mould fermentation products in addition to being of historical importance. A great deal of attention has been devoted to the study of the conditions for their production, particularly in the case of citric acid, on account of its commercial importance. The relative amounts of the acids produced are intimately connected with the composition of the inorganic constituents of the medium. Thus Molliard (1924) found that when the source of nitrogen in the nutrient salts of *A. niger* was decreased, gluconic and citric acids accumulated in the medium, whereas a deficiency of the total mineral salts led to the formation of oxalic and citric acids. Similarly Butkewitsch & Timofeeva (1935) observed that when the growth was checked by partial deprivation of combined phosphorus, sulphur or nitrogen, the yields of citric acid were high. Lack of nitrogen or phosphorus led to restriction of gluconic and oxalic acid production, but lack of sulphur increased the yield of these two acids. Citric acid almost disappeared when there was a simultaneous lack of potassium and magnesium. Deprivation of magnesium caused reduction in the yield of gluconic and oxalic acids, but these products were increased by a reduction in the potassium content of the medium. Kardo-Syssojeva (1933) using ready-grown felts of *A. niger* on 20 per cent sucrose medium obtained similar results. He observed that decrease in the total salts or addition of calcium carbonate favour gluconic acid production, but increase of nitrogen salts stimulates the production of gluconic acid in acid media but decreases it in presence of calcium carbonate. Sotnikov (1934), presenting the nitrogen in the form of magnesium nitrate, found that the production of citric acid varied hyperbolically with the concentration of magnesium nitrate. It was immaterial whether the nitrogen was present as nitrate or ammonium ion, but the

specific action of magnesium was exerted only in presence of nitrate, and then only with active strains. Bonnet & Jacquot (1935) conclude that the formation of oxalic acid by *A. niger* increases with age of culture in media containing potassium nitrate, but that none is formed in media containing ammonium salts. They are of the opinion that oxalic acid is an unusable by-product, and that its production is related to the maintenance of an appropriate energy balance in the culture, whereas citric acid is formed from spores and is subsequently utilized during growth of the mycelium. It is difficult on this hypothesis to account for the large yields of citric acid obtained from non-sporing cultures.

The unfavourable action of ammonium salts on the citric acid fermentation had previously been observed by Porges (1932*b*). He found that sodium nitrate gave better yields than ammonium nitrate or chloride, and that iron and zinc were essential for rapid growth and production of citric acid, but Chrzaszcz & Peyros (1935) found that zinc salts inhibited the citric acid fermentation and recommended a medium containing ammonium nitrate as source of nitrogen. The source of oxalic acid is considered by Butkewitsch (1934) to be the mycelium. Citric acid is produced not only from sugars but also from acetic, succinic and aconitic acids, and from ethyl alcohol (Bernhauer & Scheuer, 1932; Bernhauer & Böckl, 1932).

Glycollic and glyoxylic acids. These acids were shown by Bernhauer & Scheuer (1932) to be produced by many strains of *A. niger* from acetic acid (as the calcium or sodium salt). The glycollic acid rapidly disappeared and gave place to glyoxylic acid which was also produced concurrently. Since sodium acetate gives more oxalic acid than sodium glycolate, and sometimes more than the succinate, they concluded that acetic acid is converted into oxalic acid by way of succinic, not by way of glycollic acid. Succinic acid may be converted not only into oxalic but also into citric acid.

Fumaric acid. The first record of the formation of fumaric acid by moulds is due to Ehrlich (1911), who found it amongst the products of *Mucor stolonifer* when grown on glucose and fructose solutions. Wehmer (1918) isolated a strain of *Aspergillus* closely allied to *A. niger*, capable of producing fumaric acid from sucrose in 60–70 per cent yield. He named the species *A. fumaricus*.

Wehmer later (1928) reported that the organism from which he had previously obtained fumaric acid in quantity had lost its power of yielding the acid, but that in its place considerable amounts of gluconic acid, together with some citric and malic acid, were formed. Thies (1930) was able to restore the fumaric acid production to some extent by aerating the glucose substrate with oxygen. In this manner he obtained a heavy submerged growth yielding some fumaric acid. In an investigation of *Rhizopus* species, Takahashi & Asai (1927) showed that fumaric acid was a product of various members of this genus, and the formation of the acid has also been noted by Gottschalk (1926) who obtained it from pyruvic acid by the agency of *Mucor stolonifer* (*Rhizopus nigricans*). A *Penicillium* species, *P. griseo-fulvum* Dierckx, also yields fumaric acid in small amount (Raistrick & Simonart, 1933).

Malic acid. This acid has recently been recorded by several observers as a mould product, but it seems to occur normally only in small amount and not as a major

product of fermentation. Wehmer (1928) showed that it is obtained from sucrose by the action of *Aspergillus fumaricus*, Birkinshaw *et al.* (1931, ix) recovered it from the metabolic products of a white species of *Aspergillus*, and Birkinshaw & Raistrick (1931, xvii) from a species of *Clasterosporium* when grown on glucose. It is claimed as a product of *Aspergillus niger* by Bernhauer *et al.* (1932). Recently Yuill (1936) has described a fermentation of sugar in presence of chalk by *A. flavus* Link, in which a subsurface growth is attained. Under these conditions malic acid is produced in considerable yields and is accompanied by smaller quantities of succinic and fumaric acids.

Succinic acid. This acid was recognized as a metabolic product of *Mucor mucedo* by Fitz (1873) and of *M. racemosus* by Emmerling (1897). More recently Takahashi & Asai (1933) found that four out of five species of *Mucor* examined produced succinic acid along with other products from glucose when calcium carbonate was present in the medium. It seems to be formed in small amount by a variety of organisms. Thus Birkinshaw *et al.* (1931, ix) and Birkinshaw & Raistrick (1931, xvii) showed that it is formed from glucose by a white species of *Aspergillus*, by a species of *Clasterosporium* and by *Fumago vagans*. It was also obtained from *Aspergillus terreus* (Raistrick & Smith, 1935) and *Penicillium aurantio-virens* Biourge (Birkinshaw & Raistrick, 1932).

The origin of the succinic acid is obscure. From the small yields usually obtained it might be derived from the breakdown of the cell proteins as is believed to be the case in yeast fermentation, but there is no reason to suppose that it could not equally well be formed by oxidative breakdown of sugar, particularly as it is closely related chemically to other simple acids, such as fumaric and malic acid. Since fumaric acid has been obtained in large yield it is improbable that it arises by way of the mould protein and not in some more direct manner.

Aconitic and itaconic acids. These two acids are related chemically to citric acid, since they may be derived from it by heating. Loss of a molecule of water from citric acid affords aconitic acid, and loss of a molecule each of water and carbon dioxide yields itaconic acid. Bernhauer & Böckl (1932) found that one strain of *Aspergillus niger* produced citric acid in over 20 per cent yield from aconitic acid, and was also able to convert citric into aconitic acid. Oxalic acid and reducing substances were formed in addition. The production of itaconic acid together with gluconic and citric acids was observed by Kinoshita (1931), using sucrose or fructose but not glucose as source of carbon. The organism, which he named *A. itaconicus*, was found in the expressed juice of pickled plums. When the source of nitrogen was changed, ammonium nitrate replacing potassium nitrate, the production of mannitol was noted. The author suggests that the sequence of formation of products is sugar → gluconic acid → citric acid → aconitic acid → itaconic acid.

Lactic acid. Lactic acid has long been recognized as a frequent product of bacterial fermentation, but has only recently been claimed as a product of moulds. Takahashi & Asai (1933) found that species of *Mucor* gave rise to traces of lactic acid when grown on glucose, and Kanel (1934) obtained from a *Rhizopus* species related to *R. japonicus* a 40 per cent yield of lactic acid on a medium containing 10 per cent of sugar and 4 per cent of chalk.

Ethyl alcohol. Whilst moulds in general give rise to an oxidative type of fermentation, it will be shown that some species at any rate afford the same products as yeast, in yields comparable with those obtained in an alcoholic fermentation. The production of ethyl alcohol by species of *Mucor* has frequently been observed; it was reported as early as 1876 by Fitz using *M. racemosus*. Until recently, however, it was thought that production of ethyl alcohol by species of *Aspergillus* and *Penicillium* was exceptional, although its production was recorded by Elfving (1890) and Kostytshev & Afanassjewa (1922) using "*P. glaucum*", by Sanguineti (1897) using *Aspergillus oryzae* and by Kostytshev (1907) using *A. niger*. However, Birkinshaw *et al.* (1931, III) and Birkinshaw *et al.* (1931, IV) prepared balance sheets for ninety-six species or strains of *Aspergillus* and seventy-five species of *Penicillium*, and found that ethyl alcohol is a frequent product of metabolism of species belonging to these genera, and is a characteristic of certain groups of related species, whilst it is not produced by other groups. Thus the *Aspergillus flavus-oryzae* group regularly produces alcohol, but none was found in the products of the *A. glaucus* group. Ethyl alcohol was also shown to be produced from glucose by other species from widely differing genera, e.g. *Eidamia viridescens* and *E. catenulata*, *Trichospora lignorum* and *Helminthosporium geniculatum* (Birkinshaw *et al.* 1931, VI).

The genus *Fusarium* may, however, be claimed as the alcohol-former par excellence. Anderson & Willaman (1922) found that ethyl alcohol was produced by *F. lini* in yields comparable with those from yeast, and other observers have drawn attention to the similarity of the mechanism to that of a yeast fermentation. Birkinshaw *et al.* (1931, V) showed that alcohol was a product of all the species of *Fusarium* examined, only quantitative differences being found in the products.

Acetaldehyde. Using sulphite as interceptor, Cohen (1920) and Neuberg & Cohen (1921) obtained acetaldehyde from glucose by the action of various moulds, including species of *Monilia*, *Mucor*, *Oidium*, *Aspergillus* and *Penicillium*. More recently Bernhauer & Thelen (1932) reported that in presence of sodium sulphite, acetaldehyde is produced from sucrose in up to 60 per cent of the theoretical yield by *Aspergillus niger*. In the absence of sulphite, oxalic and citric acids are formed, but when acetaldehyde is obtained these acids are absent from the fermentation products. Thus with moulds as with yeast the presence of sulphite causes a profound modification of the fermentation process. In the case of some moulds which give rise to alcoholic fermentation, e.g. *Helminthosporium geniculatum* and *Clasterosporium*, acetaldehyde can be isolated in small amounts without any interceptor present (Birkinshaw & Raistrick, 1931, XVII).

Ethyl acetate. Ethyl acetate is formed when *Penicillium digitatum* Saccardo (= *P. olivaceum* Wehmer) is grown on glucose solution (Birkinshaw *et al.* 1931, XVIII). This seems to be the only record of this substance as a product of mould fermentation. Whilst no definite evidence as to its mode of origin is forthcoming, it is suggested that it may arise by a modified Cannizzaro reaction from acetaldehyde.

Methyl glyoxal. This substance, which in the Neuberg scheme of alcoholic fermentation (now discarded) was regarded as the first stage in the breakdown of

hexose, is actually produced by the action of *Aspergillus niger* on sodium hexose-diphosphate in presence of toluene according to Suthers and Walker (1932).

Pyruvic and dimethylpyruvic acids. Pyruvic acid, of which methyl glyoxal is the aldehyde, has been recently obtained by Hida (1935) as a fermentation product of *A. niger* from glucose or sucrose in presence of sodium sulphite. Hida isolated both pyruvic and dimethylpyruvic acids, and found that the presence of ammonium salts inhibited the production of the former and favoured that of the latter acid.

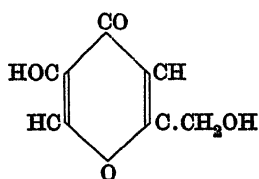
Glycerol. Glycerol is another product of yeast fermentation which is probably a fairly common metabolic product of moulds. Emmerling (1897) obtained it from *Mucor racemosus* on sucrose. Birkinshaw *et al.* (1931, IX) and Birkinshaw & Raistrick (1931, XVII) showed that it was produced from glucose by a white species of *Aspergillus*, *Helminthosporium geniculatum*, a *Clasterosporium* and *Aspergillus Wentii*.

Erythritol, trehalose and mannitol. Inactive erythritol has recently been isolated from the mycelium of *Penicillium brevi-compactum* Dierckx and of *P. cyclopium* Westling. It was obtained in small yield together with mannitol when growth was stopped before all the glucose had disappeared (Oxford & Raistrick, 1935). Erythritol was found in largest amounts in the earlier stages of fermentation. Mannitol has frequently been recorded in the mycelium of various species of *Aspergillus* and *Penicillium* together with trehalose, but has been regarded as a reserve food product rather than a true fermentation product. Birkinshaw *et al.* (1931, IX) and Birkinshaw & Raistrick (1931, XVII), however, were able to isolate yields of mannitol up to 50 per cent of the sugar utilized from the metabolism solution of various species of *Aspergillus*, *Penicillium*, a *Helminthosporium* and a *Clasterosporium* grown on glucose media. Mannitol is also the main metabolic product of *Byssoschlamys fulva* Olliver & Smith (Raistrick & Smith, 1933). Coyne & Raistrick (1931) tested various sugars as a source of mannitol production by a white species of *Aspergillus*. They found that the hexoses mannose, galactose and glucose and the pentoses arabinose and xylose afforded mannitol, but that fructose which readily yields mannitol by chemical reduction is not converted by the mould into mannitol. The reaction obviously does not depend on a mere chemical reduction of a carbonyl group of a sugar, but involves more complex changes which await investigation.

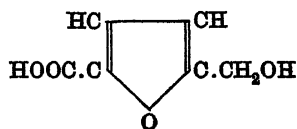
Mannonic and glycuronic acids. *d*-Mannonic acid was obtained by Angeletti & Cerruti (1930) in 9 per cent yield when *Penicillium purpurogenum* var. *rubrisclerotium* was grown on a synthetic medium containing *d*-mannose. This organism was employed by May *et al.* (1929) for the semi-large-scale production of *d*-gluconic acid from glucose. From the evidence already obtained with reference to gluconic acid, and from the similarity of the two reactions, it appears probable that *d*-mannonic acid is formed directly by a simple oxidation of mannose. Itto (1933) obtained glycuronic acid together with gluconic, fumaric, malic and succinic acids, as a fermentation product of *Penicillium* sp.

Kojic acid and Sumiki's acid. Kojic acid, $C_6H_6O_4$, was first isolated from the mycelium of *Aspergillus oryzae* by Saito (1907), but it was left to Yabuta (1912) to investigate the substance in greater detail. He later (1924) determined the constitution of kojic acid as a 5-hydroxy-2-hydroxymethyl- γ -pyrone. Yabuta obtained

kojic acid from several species of *Aspergillus*, but not from any *Penicillium* species. Wijkman (1924), using a strain of *Aspergillus*, obtained kojic acid from sucrose and claims that the acid is only produced in paraffined or quartz vessels, but not in untreated glass flasks. Kinoshita (1927), employing a sucrose medium with cobalt purpureo-chloride (a cobaltammine) as source of nitrogen, obtained a 33 per cent yield of kojic acid. May *et al.* (1931) were able to increase the yield from glucose to 45 per cent of the glucose present and 55 per cent of that consumed under the most favourable conditions. Katagiri & Kitahara (1933) claim yields of 63–66 per cent of kojic acid from *A. oryzae* when grown on 5–20 per cent glucose solutions at a pH of 2.1. The production of kojic acid was shown by Birkinshaw *et al.* (1931, VII) to be diagnostic for the *flavus-oryzae-tamaris* group of *Aspergillus* provided certain simple conditions as to composition of medium and temperature of incubation are observed. The test for kojic acid is easily performed, since the acid gives an intense blood-red colour with ferric chloride. Kojic acid has also been obtained from a *Penicillium*, namely *P. Daleae* Zaleski. The acid is markedly toxic to dogs, rabbits and rats (Friedemann, 1934). The similarity of the structure of kojic acid, which is a γ -pyrone, to the pyranose form of the glucose molecule suggests a close



Kojic acid



Sumiki's acid

relationship, but the hypothesis that kojic acid originates directly from glucose, on the lines of the chemical synthesis of kojic acid by Maurer (1930) is somewhat discounted by the fact that kojic acid is produced from a variety of substrates which include the pentoses xylose and arabinose (Challenger *et al.* 1929).

Sumiki (1929) found in the metabolic products of *Aspergillus glaucus* grown on glucose or sucrose media 2-hydroxymethylfuran-5-carboxylic acid ($C_6H_6O_4$) and later (1931) reported that this acid was also produced by *A. clavatus*, *A. niger*, *A. oryzae*, and *A. Wentii*.

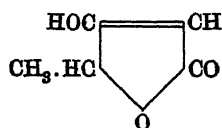
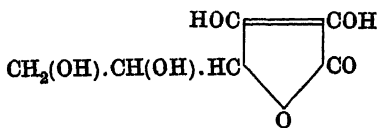
Sumiki's acid bears much the same relationship to the furanose sugars that kojic acid bears to those having the pyranose structure.

The products so far considered have all contained not more than six atoms of carbon, and therefore might conceivably be produced by breakdown or rearrangement of a single hexose molecule. This by no means exhausts the possibilities of moulds since many products containing more than six carbon atoms have been isolated in the pure state and testify to the powers of synthesis of mould fungi. It will be convenient to consider first the non-benzenoid ring compounds.

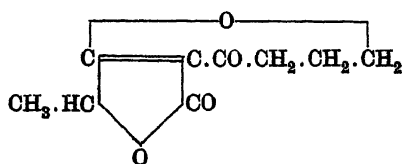
(2) Non-benzenoid ring compounds

Penicillium Charlesii acids. *P. Charlesii*, an organism isolated from spoiled maize, in the hands of Clutterbuck *et al.* (1934), and Clutterbuck *et al.* (1935) has

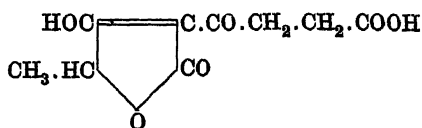
been shown to yield a series of related acids, viz. γ -methyltetronic acid, carolic acid, carolinic acid, carlic acid and carlosic acid. The four last-named acids may be regarded as butyrolactone, succinic and butyric acid derivatives of γ -methyltetronic acid and γ -tetronylacetic acid. A most interesting point about the structure of these acids is their close relationship to ascorbic acid (vitamin C), a relationship which is borne out by the similarity of the absorption spectra (Herbert & Hirst, 1935).

 γ -Methyltetronic acid

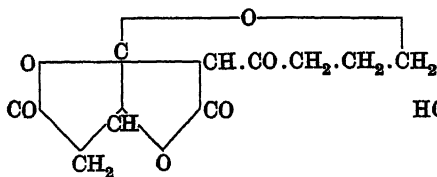
Ascorbic acid



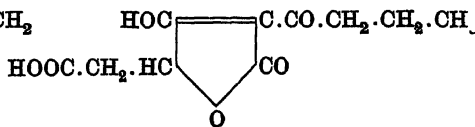
Carolic acid



Carolinic acid



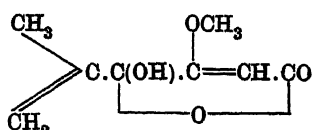
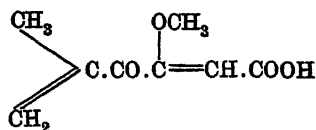
Carlic acid



Carlosic acid

Terrein. Two out of five strains of *Aspergillus terreus* Thom were shown by Raistrick & Smith (1935) to yield a new metabolic product, $C_8H_{10}O_3$, which they named terrein. Whilst the structure of this substance is not completely elucidated it is undoubtedly a hydroxycyclopentanone derivative and represents the first example of synthesis of a cyclopentane ring by moulds.

Penicillic acid. Penicillic acid was first isolated by Alsberg & Black (1913) from *Penicillium puberulum* Bainier, an organism isolated from mouldy maize. They determined the empirical formula, $C_8H_{10}O_4$, of the anhydrous acid, described its chemical properties and found that it was markedly toxic to mice. Birkinshaw *et al.* (1936), in reinvestigating the acid, found that the yield obtainable from Alsberg & Black's organism was very small and rapidly diminished over a period



Penicillic acid

of 3 years until none of the product was obtained, but fair yields of the acid were obtained from *P. cyclopium* Westling. The acid has the constitution γ -keto- β -methoxy- δ -methylene- Δ^{α} -hexenoic acid or the corresponding hydroxylactone.

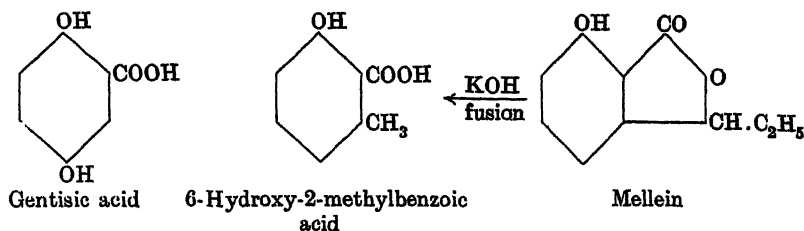
The presence of a methoxy group is interesting, as although this group is of frequent occurrence in benzenoid products of moulds, this is the only example so far noted of its presence apart from the aromatic nucleus.

(3) Benzenoid compounds

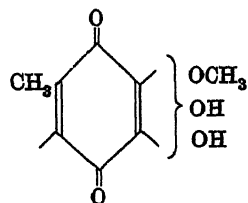
Methylsalicylic acid, gentisic acid and mellein. 6-hydroxy-2-methylbenzoic acid $C_8H_8O_3$ was obtained by Anslow & Raistrick (1931) as a metabolic product of *Penicillium griseo-fulvum* Dierckx when grown on a Czapek-Dox glucose medium. The presence of the acid in the metabolism solution is easily recognized, since it yields an intense purple colour with ferric chloride. It is also formed by *P. flexuosum* Dale (Oxford *et al.* 1935).

The same organism which produces methylsalicylic acid, namely *P. griseo-fulvum* Dierckx, was shown by Raistrick & Simonart (1933) to afford gentisic acid (2:5-dihydroxybenzoic acid), giving a deep blue colour with ferric chloride.

Mellein, $C_{10}H_{10}O_3$, a metabolic product of *Aspergillus melleus* Yukawa, was first isolated and described by Nishikawa (1933), and later Yabuta & Sumiki (1933, 1934) obtained the same product from *A. ochraceus* and brought the final proof of its constitution as that of a lactone of 6-hydroxy-2- α -hydroxypropylbenzoic acid. It is obviously closely related to 6-hydroxy-2-methylbenzoic acid, into which it is converted on potash fusion.



Methoxydihydroxytoluquinone. This product, $C_8H_8O_5$, was shown by Birkinshaw & Raistrick (1931, XII) to be produced by strains of *Penicillium spinulosum* on Czapek-Dox glucose. The quinone forms purple-black crystals, yields a deep brown colour with ferric chloride, and gives alkali salts which are of an intense purple colour in aqueous solution. The constitution has not been completely determined, but it appears to be a substituted *p*-toluquinone, containing one methoxyl and two hydroxyl groups. This is the first recorded instance of the production by moulds of a benzoquinone derivative.

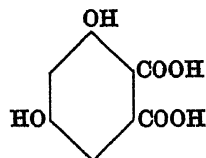
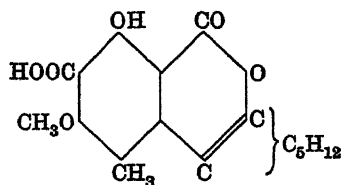
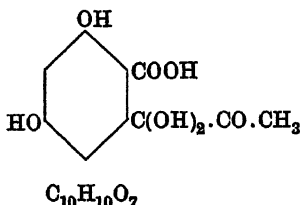
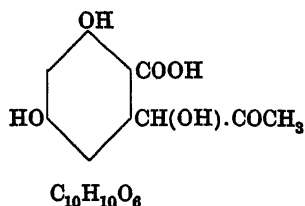
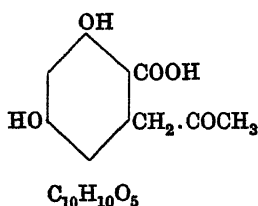


Puberulic acid, $C_8H_6O_8$ and related acid, $C_8H_4O_8$. These two substances described by Birkinshaw & Raistrick (1932) occur together as metabolic products of *Penicillium puberulum* Bainier and of *P. aurantiovirens* on glucose media. They both give deep brown colorations with ferric chloride. Puberulic acid, $C_8H_6O_8$, is a colourless substance bearing some resemblance to a dihydroxybenzenedicarboxylic acid, but it was shown not to have that constitution. Although the constitution has not as yet been fully determined,

the most probable structure is that of an aldehydotrihydroxybenzoic acid. The substance $C_8H_4O_6$ is yellow in colour and was shown by Barger & Dorrer (1934) to be a quinonoid body related to puberulic acid.

Products of Penicillium brevi-compactum. Several related benzenoid compounds have been obtained from this organism which is frequently found on spoiled maize and is pathogenic to honey bees. These substances, which were described and characterized by Clutterbuck *et al.* (1932), Clutterbuck & Raistrick (1933) and Oxford & Raistrick (1932, 1933*a*) may all be regarded as derivatives of 2:4-dihydroxybenzoic acid.

Mycophenolic acid was probably first encountered by Gosio (1896), who obtained it from a species to which he gave the name of "*P. glaucum*", and was later observed by Alsberg & Black (1913) to be formed by *P. stoloniferum* Thom. The acid is readily detected owing to the violet colour produced in aqueous solution by the action of ferric chloride. Gosio, on the basis of a single combustion, assigned to mycophenolic acid the constitution $C_9H_{10}O_8$, but this was corrected by Alsberg & Black to $C_{17}H_{20}O_6$. The constitution of mycophenolic acid is, so far as it is known, shown below, together with the constitution of the other *P. brevi-compactum* acids, which have the empirical formulae $C_{10}H_{10}O_5$, $C_{10}H_{10}O_6$, $C_{10}H_{10}O_7$ and $C_8H_6O_6$.

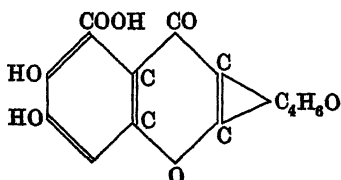


The order of the appearance of these products in the fermentation was examined by Oxford and Raistrick (1933*c*) in order to throw some light on the mechanism involved in their formation. The yields of mycophenolic and 3:5-dihydroxyphthalic acids increased continuously during the whole course of the metabolism. The acid $C_{10}H_{10}O_7$ arose mainly toward the end of the incubation period. The yield of the acid $C_{10}H_{10}O_6$ increased rapidly to a maximum, then decreased and finally disappeared entirely. The acid $C_{10}H_{10}O_5$ was present in the early stages of metabolism but disappeared in the later stages. It was concluded that 3:5-dihydroxyphthalic

acid is an oxidation product of one or more of the other products, and that the acid $C_{10}H_{10}O_7$ arises by direct oxidation of $C_{10}H_{10}O_6$. The acid $C_{10}H_{10}O_6$ may be formed by reduction of the acid $C_{10}H_{10}O_8$, which may also be the precursor of mycophenolic acid. No indication was obtained of the mechanism involved in the initial formation of the resorcinol nucleus from glucose.

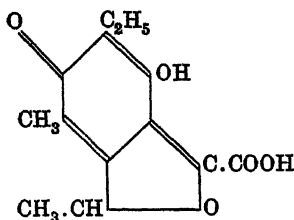
(4) Pigments

Citromycetin and citrinin. Citromycetin, $C_{14}H_{10}O_7 \cdot 2H_2O$, produced on a modified Czapek-Dox-glucose medium by strains of *Citromyces glaber* Wehmer, was first isolated and described by Hetherington & Raistrick (1931, XI). Citromycetin forms lemon yellow needles and gives an intense olive green colour with ferric chloride. The constitution assigned to it is:



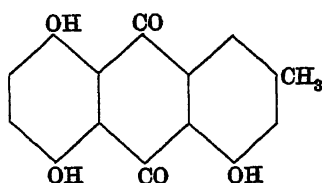
The nature of the grouping C_4H_5O had not as yet been determined, but the acetone which is formed on alkaline hydrolysis of citromycetin must arise from this portion of the molecule. Citromycetin is specific for certain strains of *Citromyces glaber* Wehmer (now named by Thom *Penicillium (Citromyces) glabrum* Wehmer), since it is only given by some (not all) strains of this particular species, and it is suggested that its production by any organism under examination definitely allocates that organism to the genus *Citromyces*.

Citrinin, also isolated, described and characterized by Hetherington & Raistrick (1931, XIV) is another yellow crystalline pigment, which, however, gives an iodine-brown colour with $FeCl_3$. It is a product of *Penicillium citrinum* Thom on the modified Czapek-Dox glucose medium. From a study of its breakdown products Coyne, Raistrick & Robinson (1931, xv) assigned to it the quinonoid structure:

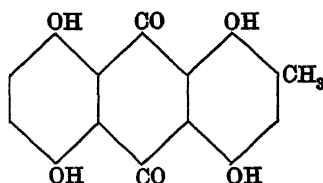


Helminthosporium pigments. The mycelium of *Helminthosporium gramineum* Rabenhorst, grown on a medium containing glucose as sole source of carbon, contains considerable amounts (30 per cent) of crystalline hydroxyanthraquinones consisting of a mixture of helminthosporin, $C_{15}H_{10}O_5$, and catenarin, $C_{15}H_{10}O_6$ in the proportion of two to three parts of the former to one part of the latter (Charles

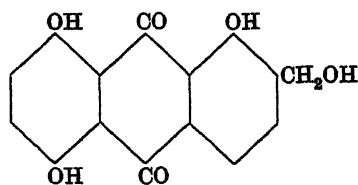
et al. 1933). Raistrick *et al.* (1933 *a, b*, 1934) showed that the mycelium of *H. cynodontis* Marignoni and *H. euchlaenae* Zimmermann, when grown under the same conditions, contains another hydroxyanthraquinone, cynodontin, $C_{15}H_{10}O_6$, which is accompanied in the case of *H. cynodontis* by small amounts of helminthosporin. Catenarin is produced in almost pure form by *H. catenarium*, but is associated in the mycelium of *H. velutinum* with appreciable amounts of ergosterol. *H. tritici-vulgaris* Nisikado yields catenarin together with smaller amounts of tritisporin, $C_{15}H_{10}O_7$. *H. velutinum* Link and *H. avenae* Eidam afford respectively catenarin and cynodontin accompanied in each case by ergosterol. It is an interesting fact that all the species of *Helminthosporium* examined which produce hydroxyanthraquinones are placed by Nisikado (1929) in the subgenus *Cylindo-Helminthosporium*, whereas those species almost or completely devoid of pigments fall into the subgenus *Eu-Helminthosporium*. The following constitutions are assigned to these pigments:



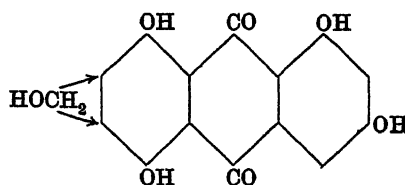
Helminthosporin



Cynodontin



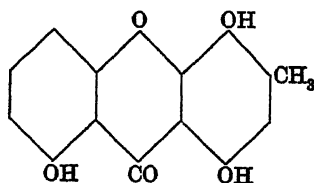
Catenarin



Tritisporin

In tritisporin the position of the CH_2OH is uncertain, but it must occupy one or other of the two positions indicated.

Ravenelin. Ravenelin, $C_{14}H_{10}O_5$, is a crystalline methyltrihydroxyxanthone pigment obtained from the mycelium of *H. Ravenelii* Curtis, and, in smaller yields, from *H. turcicum* Passerini, when grown on Czapek-Dox glucose medium (Raistrick *et al.* 1936). These two species belong to the subgenus *Eu-Helminthosporium*. The constitution of ravenelin is probably the following:



Aspergillus glaucus pigments. Gould & Raistrick (1934) isolated three crystalline pigments from species of the *A. glaucus* series. More than 40 per cent yields

of crude pigments may be obtained from the dried mycelium. Flavoglucin, $C_{19}H_{28}O_8$, is probably derived from the perithecia and is elaborated by all the twenty-five species examined. Auroglucin, $C_{19}H_{22}O_8$, was obtained from fifteen species and rubroglucin, $C_{18}H_{12}O_5$, from only four species. Although various coloured species of *Aspergillus* belonging to other groups were examined, none gave rise to the *glaucus* pigments which thus appear to be specific for that series. On the basis of these results the *A. glaucus* series has been divided into three distinct pigment groups. The constitution of these pigments has not yet been elucidated, but flavoglucin and auroglucin are closely related and appear to be carotenoid in nature.

β -carotene. Schopfer (1935*b*) noted that when *Mucor Hiemalis* or *Phycomyces Blakesleeanus* were grown on media containing asparagine or glycine as the source of nitrogen, they synthesized β -carotene, which was identified by its absorption spectrum. The synthesis of carotene occurred to a greater extent in mycelia of the (+) sex. Curiously enough its formation was inhibited by the presence of 1 mg. of vitamin B₁ per litre in the culture medium. This observation is of considerable significance in view of the fact that β -carotene is the precursor of vitamin A.

Fulvic acid. This substance was obtained by Oxford *et al.* (1935) as a metabolic product of *Penicillium griseo-fulvum* Dierckx, when grown on glucose solutions with ammonium salts as source of nitrogen. It is a yellow crystalline phenolic acid, $C_{14}H_{12}O_8$, giving a deep green colour with aqueous ferric chloride. Fulvic acid is obtained in larger yield from *P. flexuosum* Dale and *P. Brefeldianum* Dodge, the greater part of the fulvic acid being found in the mycelium in the case of the latter organism. The structural formula of the acid is still under investigation.

Chrysogenin. This yellow pigment occurs associated with a protein in the metabolism solution of *P. chrysogenum* Thom when grown on a glucose medium (Clutterbuck *et al.* 1932). The most probable empirical formula of chrysogenin is $C_{18}H_{22}O_6$. It is micro-crystalline, has a remarkably high optical rotation, $[\alpha]_{5461} - 762^\circ$, yields an olive brown colour with aqueous ferric chloride, and probably contains a quinol nucleus.

Respiratory pigment. An alkaline extract of *Aspergillus niger* spores was found to display maximum absorptions at 2840, 4300, and 4500 Å. Giordani (1934) is of the opinion that this indicates the presence of a respiratory flavin-like pigment.

Monascorubrin and monascoflavin. Monascorubrin, $C_{22}H_{24}O_5$, is a red crystalline pigment isolated by Nishikawa (1932) from the mycelium of *Monascus purpureus* Went. It is converted by hydrogen peroxide into a yellow pigment monascoflavin, $C_{17}H_{22}O_4$, which can also be obtained directly from the aged mycelium of *M. purpureus*.

Oosporin and aurantin. Oosporin is a colourless crystalline product of empirical formula $C_{10}H_{14}O_2$, giving a purple brown colour with $FeCl_3$. Aurantin, a yellow crystalline pigment, $C_{16}H_{22}O_8$, gives a similar colour to oosporin with ferric chloride. Both substances were obtained by Nishikawa (1934) from the mycelium of *Oospora aurantia* (Cooke) Sacc. et Vogl.

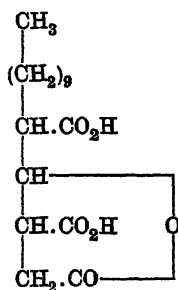
Two pigments were isolated by Sutter & Wijkman (1933) from *Penicillium*

glaucum, together with the glauconic acids described later. One of these is a yellow crystalline substance, $C_{19}H_{22}O_4$, soluble in light petroleum, the other is red in colour and contains nitrogen.

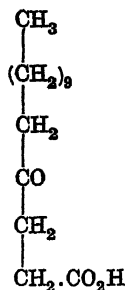
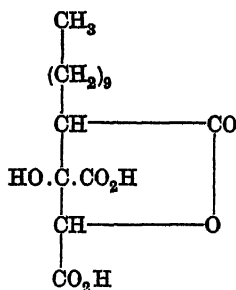
(5) Miscellaneous products

Spiculisporic and minioluteic acids. These substances are crystalline monolactones of hydroxylated tribasic aliphatic acids containing 17 and 16 carbon atoms respectively. Spiculisporic acid, $C_{17}H_{28}O_6$, the lactone of γ -hydroxy- $\beta\delta$ -dicarboxypentadecanoic acid, was isolated, together with small amounts of γ -ketopentadecanoic acid, from the metabolic products of *P. spiculisporum* Lehman by Clutterbuck *et al.* (1931, xvi). The organism was grown on a Czapek-Dox glucose medium. On oxidation with potassium permanganate in acetone solution, spiculisporic acid gives an almost quantitative yield of γ -ketopentadecanoic acid, so that the possibility of this latter acid being an artefact cannot be entirely ruled out. Spiculisporic acid is also elaborated by *P. crateriforme* Gilman and Abbot (Oxford & Raistrick, 1934).

Birkinshaw & Raistrick (1934) showed that *P. minio-luteum* Dierckx is not only able to convert glucose into spiculisporic acid, but that minioluteic acid, the γ -lactone of $\alpha\beta$ -dihydroxy- $\beta\gamma$ -dicarboxy-*n*-tetradecanoic acid is also formed by this organism in equal or larger amount. The constitutions of these three acids, as determined by the authors cited, are the following:

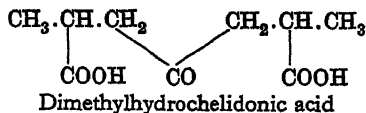


Spiculisporic acid

 γ -Ketopentadecanoic acid

Minioluteic acid

Glauconic acids and byssochlamic acid. From a strain of "*P. glaucum*", Wijkman (1931) isolated amongst other products two colourless crystalline acids: glauconic acid I, $C_{18}H_{20}O_7$ and glauconic acid II, $C_{18}H_{20}O_6$. The names have since been changed to glauconic acid and glaucanic acid respectively. Glauconic acid, on heating, affords $\alpha\beta$ -diethylacrolein, $C_7H_{12}O$, and another fission product glauconin, $C_{11}H_8O_6$ (Sutter & Wijkman, 1933, 1935) which with aqueous hydrochloric acid at 200° losses 2 molecules of CO_2 and yields a dimethylhydrochelidonic acid $C_9H_{14}O_5$. Glauconic acid is also produced from sucrose by a *Penicillium* parasitic on *Aspergillus niger* (Yuill, 1934). Glaucanic acid appears to resemble closely byssochlamic acid, isolated by Raistrick & Smith (1933) from the metabolic products of *Byssochlamys fulva* Olliver & Smith. These two



acids, which are isomeric but not identical, both appear to contain two stable acid anhydride groupings, since they both titrate as tetrabasic acids although containing only six oxygen atoms, and no active hydrogen atoms by the Zerewitinoff method. The tetracarboxylic acid form cannot be isolated, only the anhydride forms being recovered on acidifying a neutralized solution. These acids are still under investigation (cf. Sutter & Wijkman, 1936).

Glaucic acid. This acid described by Sumiki (1933) is a product of *Aspergillus glaucus*. It is a monobasic acid, $C_{17}H_{22}O_5$, containing no hydroxyl or methoxyl groups.

Palitantin. Palitantin, $C_{14}H_{22}O_4$, is an unsaturated dihydroxyaldehyde, isolated by Birkinshaw & Raistrick (1936) from the metabolism solution of *Penicillium palitans* Westling, when grown on a Czapek-Dox glucose medium.

(6) Sterols

The earlier observers who claim to have isolated sterols from moulds probably obtained impure products since the methods of separation were at that time crude and inefficient. Gérard (1892, 1895) obtained a sterol from *Penicillium glaucum* on Raulin's medium, and another from *Mucor mucedo* on lactose. Rémond & Lassalle (1925) reported the production of "cholesterol" by *Penicillium glaucum* and Gayral (1926) the formation of phytosterol by *Aspergillus niger*. A *Mucor* in the hands of Reindel & Walter (1926) afforded a sterol identical in properties with ergosterol from yeast. Heiduschka & Lindner (1929) determined the ergosterol content of *Dematium pullulans*, *Penicillium glaucum* and *Aspergillus oryzae* as 0.30, 0.75, and 0.46 per cent respectively of the dry matter. Sumi (1928, 1929) isolated 0.08 per cent of crude ergosterol from the spores of *A. oryzae*, whereas Takata (1929) obtained a yield of 0.028 g. from the mycelium of the dried organism. Prickett *et al.* (1930) determined the most favourable conditions for the production of the sterol, and Pruess *et al.* (1931) investigated the sterol production from glucose of a number of moulds and determined the anti-rachitic potency on irradiation. The sterol formed by *Penicillium puberulum* Bainier, when grown on a Czapek-Dox medium containing glucose as sole source of carbon, was isolated and characterized by Birkinshaw *et al.* (1931). The physical properties of the sterol itself and of its benzoate were found to correspond closely with those of purified ergosterol from yeast and ergosteryl benzoate respectively, and final confirmation of its identity with ergosterol was afforded by investigation of the absorption spectrum and of the antirachitic activity acquired after irradiation. This affords direct evidence of the synthesis of ergosterol from glucose.

Pruess *et al.* (1932) and Pruess *et al.* (1932) found that the dried mycelium of *Aspergillus fischeri* and *A. oryzae* afforded ergosterol and showed that factors favouring growth of *Aspergillus*, *Penicillium* and other species were also conducive to sterol production. The latter conclusion is supported by Bernhauer & Patzelt (1935) in respect of *Aspergillus niger*. They found that the sterol production proceeds in general parallel with the formation of mycelium, and further that the yield of sterol obtained from the Ca salts of gluconic and quinic acids is only half that from

glucose. Ergosterol occurs in the mycelium of moulds not only in the free state but also as esters, and one ester has been isolated in a state of purity. Oxford & Raistrick (1933*b*) isolated crystalline ergosteryl palmitate from the mycelium of fourteen out of fifteen strains of *Penicillium brevi-compactum* Dierckx and from the mycelium of *P. italicum* Wehmer. *P. aurantio-griseum* Dierckx var. *Poznaniensis* Zaleski grown on Raulin-Thom medium was found to contain as much as 0.5 per cent of the ester.

(7) *Fats and lipins*

Although various observers have recorded the formation of fat by moulds, the biochemistry of fat production has until recently received little attention. The earliest workers in the field, Naegeli & Loew (1878) and Sieber (1881) seem to have used cultures of doubtful authenticity and purity. Fat has frequently been reported since that date in the mycelium of various fungi. Browne (1906) found 27.5 per cent of the mycelium of a *Citromyces* species to be a fat resembling butter-fat in physical and chemical properties. Belin (1926) refers to the production of fat by *Aspergillus*. Rockwell & O'Flaherty (1931) surveyed the genera *Aspergillus*, *Mucor* and *Penicillium* for fat production as part of their work on the analysis of mycelia and reported low fat contents. Barber (1927, 1929) analysed the fat of a green *Penicillium* and obtained yields of 14 per cent by growing his organism on 5 per cent sucrose for 3 months. On synthetic media containing sucrose, glucose, glycerol or xylose as sole source of carbon, the fat was apparently of the same chemical composition.

Terroine & Bonnet (1927) studied the energy relations of the production of fat from sugar by *Aspergillus niger*, and Terroine *et al.* (1927) examined the physiological significance of the ethylene linkages of mould fats. Pearson & Raper (1927) found 2.4 per cent of fat in the moist mycelium of *A. niger* and 5 per cent of fat in that of *Rhizopus nigricans*. They found that the degree of unsaturation of the fatty acids elaborated was greater in moulds grown at low temperature than in those grown at higher temperature. The effect of the mineral and organic nutrition of *Aspergillus niger* on the quality and quantity of fat produced was studied by Pontillon (1932, 1933). A similar study was undertaken by Lockwood *et al.* (1934) for the mould *Penicillium javanicum* Van Beijma, this organism being selected, since out of a number examined it gave the heaviest mats which contained a substantial fraction of fat. The best yield of fat was obtained when the medium contained a high proportion of sugar. Xylose was utilized by the mould for growth and fat production as readily as glucose. In a later paper the same team of workers (Ward *et al.* 1935) found that the mycelium contained up to 41.5 per cent of fat when a medium with 40 per cent of glucose was employed. A strain of *Aspergillus niger* was shown by Schmidt (1935) to synthesize fatty acids under conditions in which further growth could not take place. The fatty acid content of the mycelium increased by approximately 100 per cent in 3 days. This increase in fatty acid production, and the degree of unsaturation of the fatty acids themselves, were found to be unrelated to the pH of the medium. Fatty acids were formed to a greater extent under aerobic than under anaerobic conditions.

Prill *et al.* (1935) determined the factors influencing the amount and nature of the fat produced by *A. fischeri*. The fat is increased by increasing the concentration of glucose in the medium and by raising the pH of the medium (in the range 2-8). Continued incubation after all the glucose has disappeared decreases the percentage of fat and lipin phosphorus but does not affect the fatty acids.

The composition of the fat of *Penicillium javanicum* was examined by Ward & Jamieson (1934). The saturated acids isolated by hydrolysis of the oil obtained by petrol extraction of the mycelium amounted to 30.8 per cent of the total. They consisted of palmitic 69.5 per cent, stearic 28 per cent, and *n*-tetracosic acid 2.5 per cent. The unsaturated acids isolated (60.8 per cent of the total) consisted of oleic 52.1 per cent, and α - and β -linoleic acid 47.9 per cent. Kroeker *et al.* (1935) obtained from the mycelium of *P. aurantio-brunneum* ergosterol and lipins. The latter on hydrolysis yielded palmitic, stearic, oleic and linoleic acids.

A fairly complete analysis of the lipins of *Aspergillus Sydowi* was undertaken by Strong & Peterson (1934). They obtained 22 per cent of free fatty acids and a phospholipin which was separated as a complex with magnesium chloride. The fatty acids consisted of palmitic 8.8 per cent, stearic 11 per cent., *n*-tetracosic 0.9 per cent, oleic 29.6 per cent, linoleic 16.3 per cent and higher unsaturated acids 1.7 per cent. The water-soluble fraction obtained after saponification of the fats consisted of glycerol

Woolley *et al.* (1935) have examined in some detail the phospholipin fraction of *A. Sydowi*. They found that the phospholipins, amounting to 0.4-0.7 per cent by weight of the mycelium, consist of a mixture of lecithin and cephalin. On acid hydrolysis they yield glycerophosphoric acid, choline, cholamine, and oleic acid, with traces of other acids.

(8) Polysaccharides

The polysaccharide known as "spore starch" or "mould starch", which gives a blue colour with iodine has often been described. Cramer (1894) obtained it from "*Penicillium glaucum*" and Wehmer (1913) from *Aspergillus niger*, *A. fumigatus* and *Penicillium variable*. Boas (1917, 1919*a, b*, 1922) found that the polysaccharide obtained from *Aspergillus niger* is only formed on acid media at a fairly high temperature (33° C.). It is formed from various sugars, polyhydric alcohols and carboxylic acids. Mould starch was also obtained from *A. oryzae* and *A. glaucus*. Lappalainen (1919) investigated the effect of temperature and concentration of the nutrients on mould starch, and Schmidt (1925) in a similar investigation concluded that this polysaccharide is identical with amylose and therefore with isolichenin. Lipska (1927) and Hida (1934) have also studied the formation of this product. The latter author is in agreement with Boas as to the conditions necessary for starch production, but upholds the view originally advanced but later rejected by Boas, namely that starch accumulation in the strongly acid culture is due to the inhibitory action of the acid on the diastase present in the mould, rather than to the greater rate of synthesis of starch in the acid solution. The accumulation of starch would thus be a pathological rather than a physiological phenomenon. Chrzaszcz & Tiukov (1929), on the other hand, regard the formation of starch by the lower fungi as a normal process

which in some cases requires special conditions. They divide the *Penicillia* and *Citromyces* into two groups which are characterized by acid and starch production respectively.

From the mycelium of *Penicillium puberulum* Alsberg & Black (1913) obtained a substance giving a violet colour with iodine, which was considered by them to be identical with trehalum from manna. Dox & Neidig (1914*a*) isolated from the mycelium of *P. expansum* a substance, mycodextran, giving glucose on hydrolysis and no colour with iodine. Later (1914*b*) they obtained mycodextran from the mycelium of *Aspergillus* together with another carbohydrate, mycogalactan, giving galactose on hydrolysis, and no colour with iodine.

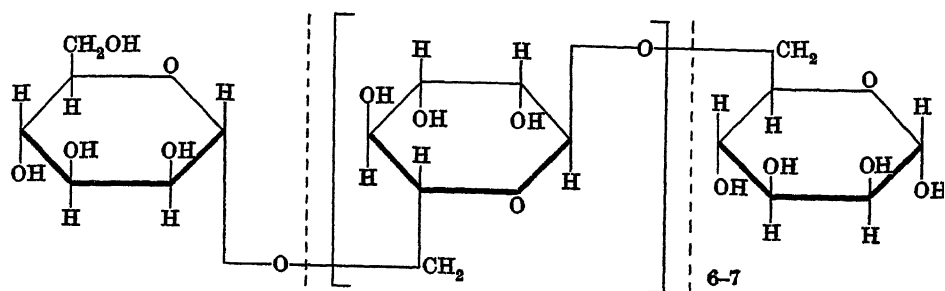
Birkinshaw *et al.* (1931, IX), Birkinshaw & Raistrick (1931, XVII) and Birkinshaw *et al.* (1931, XVIII) obtained from a white species of *Aspergillus* a polysaccharide identical with, or closely allied to, glycogen. *Fumago vagans* was shown to synthesize in fair quantity a polysaccharide, giving glucose on hydrolysis and no colour with iodine, and *Penicillium digitatum* gave a polysaccharide of high optical rotation, $[\alpha]_{5461}^{90} + 295^\circ$, likewise giving glucose on hydrolysis and no colour with iodine.

In some recently isolated polysaccharides investigation has proceeded further than mere identification of the hydrolytic fission products, and the constitution of the complex molecule has been determined. *P. Charlesii* G. Smith, when grown on Czapek-Dox glucose medium, yields two polysaccharides: mannocarolose, consisting only of mannose residues, and mannogalactose, consisting only of galactose residues (Clutterbuck *et al.* 1934). Mannocarolose has been shown by Haworth *et al.* (1935*a*) to have a chain length of 8-9 units of *d*-mannopyranose, linked through the 1 : 6 positions. This is a unique molecular structure for a polymannose.

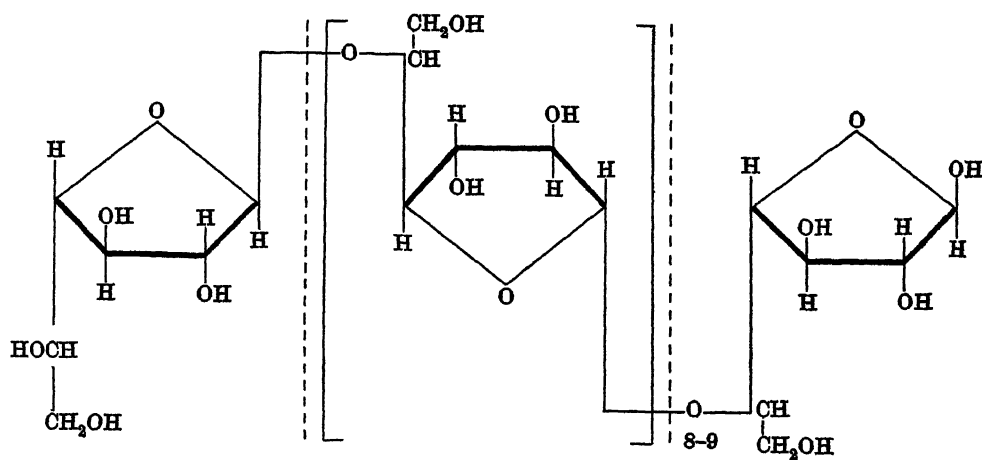
Galactocarolose is composed of a chain of about nine β -galactofuranose units linked through the 1 : 5 positions (Haworth *et al.* 1936).

Varianose, a polysaccharide obtained from *P. varians* G. Smith, yields on hydrolysis a mixture of *d*-glucose, *d*-galactose, and a third hexose which is either *l*-altrose or *d*-idose. Haworth *et al.* (1935*b*) have shown that varianose is constituted of a chain of 6-8 β -galactopyranose units with a glucopyranose unit as one end of the chain and a unit of either *l*-altrose or *d*-idose at the reducing end.

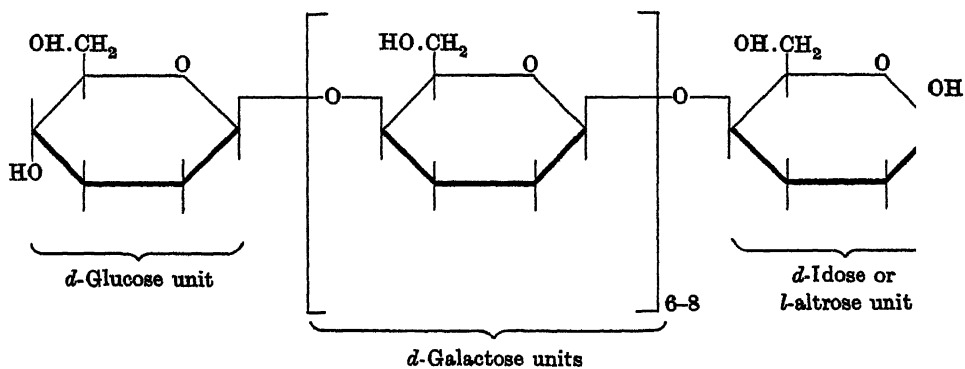
All the mould carbohydrates so far described have been neutral in reaction and have given only hexoses on hydrolysis. A product of different type was described by Raistrick & Rintoul (1931, XIII). This substance, which was named luteic acid, was obtained in 10-12 per cent yield when a non-ascosporic form of *P. luteum* Zukal was grown on a Czapek-Dox glucose medium. Luteic acid has a high molecular weight and gives rise on acid hydrolysis to glucose and malonic acid usually in the molecular ratio of about 2 : 1. On hydrolysis with dilute barium hydroxide the malonic acid is eliminated and the neutral polysaccharide luteose is produced, yielding only glucose on acid hydrolysis. Luteic acid was shown by Birkinshaw & Raistrick (1933) to be derivable not only from glucose but also from fructose, mannose, galactose, xylose, arabinose and glycerol. This affords proof of the conversion by *P. luteum* of other hexoses, of pentoses and of a trihydric alcohol into glucose.



Mannocarolose



Galactocarolose



Varianose

A careful fractionation of the total crude polysaccharide precipitate from *P. luteum* by Anderson & Raistrick (1936) revealed the fact that 8–10 per cent of a polymannose was present. Examination at different stages of growth showed that the main product is always a malonyl polyglucose, that a mannose polysaccharide is formed largely in the early stages of growth, and that it tends to be replaced by a galactose polysaccharide in the older cultures.

(9) *Compounds containing elements other than carbon, hydrogen and oxygen*

Arsenic compounds. Various cases of arsenical poisoning have been traced to the liberation of volatile arsenical products by certain moulds growing on arsenic-containing wall papers under damp conditions. This Gosio-gas, as the toxic substance was named after its discoverer, was thought by many of the earlier observers to consist of arsine. Challenger *et al.* (1933) showed that the volatile product evolved from bread cultures of *P. brevicaulis* (*Scopulariopsis brevicaulis*) containing arsenious acid, sodium methyl arsonate or sodium cacodylate is trimethylarsine. When the arsenical substrate contains an ethyl or heavier alkyl group attached to arsenic as in the case of ethylarsonic acid, $C_2H_5-AsO(OH)_2$, a mixed arsine, e.g. dimethyl-ethylarsine, is obtained. Challenger & Ellis (1935) established the formation of dimethyl-*n*-propyl-, dimethylallyl- and methyl-diethyl-arsines on bread cultures containing *n*-propylarsenic, allylarsenic and diethylarsenic acids respectively. The methylating action is not confined to arsenic since in presence of sodium selenate or selenite the mould gives rise to dimethyl selenide (Challenger & North, 1934). Thom & Raper (1932) found that the fungi producing arsenical gases are more numerous than was previously supposed, since many species or strains of *Scopulariopsis* and *Aspergillus Sydowi* afford a volatile arsenic compound which is presumably trimethylarsine.

More recently Challenger & Rawlings (1936) have shown that *Penicillium brevicaulis* converts diethyl disulphide to ethyl mercaptan and methyl ethyl sulphide, whereas di-*n*-propylsulphide yields *n*-propylmercaptan and methyl *n*-propyl sulphide. The mechanism of this curious methylating action of the mould has been considered by Challenger & Higginbottom (1935) but no definite conclusion is reached.

Chlorine compounds. *Aspergillus terreus* on Czapek-Dox glucose solution produces two crystalline chlorinated compounds which are precipitated on acidification of the metabolism solution. The probable empirical formulae are (A) $C_{16}H_{10}O_7Cl_2$ and (B) $C_{17}H_{12}O_7Cl_2$ respectively. A contains one methoxy group, titrates as a dibasic acid and is optically inactive; B contains two methoxy groups, also titrates as dibasic acid and is dextro-rotatory (Smith, 1936). The constitution still remains to be elucidated but it is noteworthy that the substances appear to bear a strong resemblance to (although they are not identical with) certain lichen products.

Nitrogen compounds. Only two crystalline nitrogenous products apart from a pigment already mentioned appear to have been recorded. Oxford *et al.* (1935) obtained from the mycelium of *Penicillium griseo-fulvum* Dierckx a colourless

crystalline nitrogenous substance, $C_{22}H_{28}O_5N_2$. The substance is an acid and yields a hydrocarbon on hydrolysis. From the mycelium of *P. Brefeldianum* Dodge a crystalline product, $C_{40}H_{70}O_5N$, was isolated. Aspergillin, the black pigment from the spores of *Aspergillus niger*, has never been obtained in crystalline form and is probably highly complex. It contains nitrogen and iron.

Proteins are elaborated by moulds and are found as constituents of the mycelium or in some cases dissolved in the metabolism solution. Clutterbuck *et al.* (1932) examined the alkali-soluble protein which is obtained in association with the pigment by acidification of the metabolism solution of *Penicillium chrysogenum*. The Van Slyke analysis showed that it closely resembled a typical alkali-soluble leaf protein. Gorcica *et al.* (1934) fractionated the nitrogenous products present in the mycelium of *Aspergillus fischeri*. They obtained 30 per cent of water-soluble non-protein nitrogen, 55 per cent of alkali-soluble protein nitrogen, and 12 per cent of alkali-insoluble residual nitrogen. The alkali-soluble protein contained one fraction precipitated by acids and a second acid-soluble fraction precipitated by copper sulphate solution. The residue contained 2.3 per cent of nitrogen of which 62 per cent was glucosamine nitrogen. Glucosamine was also identified in the hydrolysis products of *Penicillium javanicum* by May & Ward (1934). Extraction of the fat-free mycelium with boiling 10 per cent sodium hydroxide left an insoluble complex which was hydrolysed by concentrated hydrochloric acid to glucosamine hydrochloride, humus-like material and reducing nitrogen compounds.

The individual amino acid constituents of mould proteins have not been fully investigated but the proteins of *Aspergillus Sydowi* were shown by feeding experiments on rats (Gorcica *et al.* 1935*b*) to be insufficient for maintaining growth. The diet required supplements of casein, egg white or yeast protein, or better, whole wheat or maize gluten. No improvement was obtained with gelatin, cystine, histidine, tyrosine or a mixture of cystine and tyrosine. Tyrosine was actually isolated by Vorbrodts (1934) from the hydrolysis products of the protein of the mycelium of *A. niger*.

Vitamins of the B group which have already been shown to have a stimulating action on mould growth are apparently synthesized by some moulds. Takata (1929*a, b*) and Takahashi & Shoku (1929) were the first to bring evidence of the synthesis of vitamin B; the organism used was *A. oryzae*. Gorcica *et al.* (1935*a*) showed by feeding experiments that the mycelium of *A. Sydowi* grown on a synthetic medium with glucose as sole source of carbon contained the vitamins B_1 , B_2 and B_4 . The amount of mycelium required for an adequate supply of each of the three vitamins, expressed as a percentage of the total ration, was 10 per cent, 1 per cent and 30 per cent respectively. It is interesting to note that the precursors of vitamins A and D, namely β -carotene and ergosterol, are also elaborated by moulds, while certain acids obtained from *Penicillium Charlesii* are closely related in constitution to vitamin C.

VIII. ENZYMES

The subject of mould enzymes is too wide for adequate treatment in this article, and it must suffice to say that enzymes of practically every known type—oxidases, reductases, clotting enzymes, hydrolytic enzymes producing fission of polysaccharides, esterases, proteases, polypeptidases, zymase, urease, nuclease and phosphatases—are to be found in moulds. The enzymes and enzymic activities of micro-organisms were reviewed by Waksman (1922). Later work describes the preparation of various enzymes from specific organisms and the effect of variations in and additions to the medium on the nature and yield of the enzymes formed.

IX. DISCUSSION

Ample evidence has been advanced for the outstanding synthetic powers of the lower fungi, and the questions of the mechanism of the formation of the manifold products and the reason for their elaboration naturally suggest themselves.

The mechanism has been little studied except perhaps in the case of citric acid, to which reference has already been made. Two general routes are possible in the synthesis of any given metabolic product from a carbohydrate; in the first the carbohydrate molecule is assumed to be broken down to simpler molecules which are then built up into the particular product under consideration, much in the same way as proteins are first degraded in the animal body to the constituent amino acids, which are then available for synthesis of the body protein. This has been generally assumed to be the method of attack favoured by the lower fungi. The other possible method of formation of specific products consists in the primary synthesis of complex substances such as polysaccharides which are then degraded or otherwise altered by processes of reduction, oxidation, condensation and hydrolysis until the specific product is attained. The fact that a number of polysaccharides have already been isolated from moulds lends support to the hypothesis, and the close similarity between the galactofuranose residue of galactocarolose and the structure of the group of acids derived from the same organism, *P. Charlesii*, is interesting in this connexion.

Quite a number of mould metabolic products contain methoxyl groups. Browne & Phillips (1935) have recently put forward an interesting hypothesis to account for the occurrence of such groups. They suggest that methoxyl, ethoxyl and methylene dioxide groups in plant products are formed in the course of the fission of polysaccharides by processes of hydrolysis, oxidation, reduction and dehydration. In this manner the oxygen linkage in a polysaccharide may become the oxygen of a methoxyl group. Such a reaction might be expected to occur more readily if the synthesis of a polysaccharide preceded the formation of a specific mould product.

The question as to the reason for the elaboration of the various characteristic products by moulds remains unanswered. In the case of the fats and polysaccharides they may represent reserve foodstuffs stored for eventual requirements. Some products form oxido-reduction systems. Certain products have been shown to be

toxic to other organisms and their formation may represent a part of the defence mechanism of the mould. Other products again may be pathological in nature, arising from injury to the enzyme system owing to unfavourable conditions of culture. The energy of moulds is derived from oxidation of the organic nutrient, complete conversion of which into carbon dioxide and water may represent the "perfect" metabolism. In such a chain of reactions, each catalysed by its own particular enzyme, injury to any enzyme will cause the chain to be broken and will result in the accumulation of an intermediate product.

These and other problems await elucidation. Much work is still required before a coherent account of the biochemistry of moulds can be attempted, but it is hoped that in striving for this ideal much light may be thrown on the physiological processes and in particular on the synthetic activities not only of moulds but also of the whole vegetable kingdom.

X. SUMMARY

1. The study of the biochemistry of the lower fungi received a great impetus from the classical researches of Wehmer begun in 1891. The more recent developments are here reviewed. The biochemical characteristics of moulds often correspond closely with the taxonomic features and thus assist in classification. ♦

2. A medium suitable for mould growth must contain the elements sulphur, phosphorus, potassium, and (probably) magnesium. Traces of certain heavy metals, namely iron, zinc, copper, and manganese, seem to be essential for the growth of *Aspergillus niger* and probably of other moulds.

3. Combined nitrogen is also essential for growth and may be supplied in inorganic or organic form.

4. The possible sources of carbon are very diverse, alcohols, carbohydrates and many organic acids being readily utilized.

5. Although moulds are essentially aerobes, some species can tolerate a low oxygen tension. Diminution in the oxygen supply may alter considerably the products of metabolism.

6. Small amounts of certain organic substances specifically stimulate or inhibit some of the metabolic processes of moulds. Vitamins of the B group have a favourable effect on growth and sporulation in the case of some species. Certain moulds are able to elaborate substances inhibitory to other micro-organisms.

7. The metabolic products are very varied in nature and range from the simpler plant acids (oxalic, fumaric, malic and citric acids), polyhydric alcohols and products of alcoholic fermentation to substances containing non-benzenoid rings such as derivatives of furane, pyrone, tetrone acid and cyclopentanone, substances containing benzenoid rings, such as derivatives of benzene, benzoquinone, anthraquinone and xanthone, pigments, sterols, fats, phospholipins and complex polysaccharides. Vitamins of the B group and the provitamins A and D are also found amongst mould products. In presence of arsenic, certain species such as *Scopulariopsis brevicaulis* produce volatile organic arsenical compounds. Substances containing chlorine as part of the organic molecule are also synthesized from inorganic chlorides,

and organic nitrogenous compounds including proteins from inorganic nitrogen. Evidence is thus afforded for the outstanding synthetic activities of the lower fungi.

8. Whilst the mechanism of synthesis is as yet an unsolved problem, the suggestion is advanced that some of the complex products may arise from the polysaccharides which are of such frequent occurrence in mould metabolism.

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ADDENDUM

Constituents of the Medium

Steinberg (1936) has recently advanced evidence for the essential nature of molybdenum for growth and development of *Aspergillus niger*. He finds that accessory substances are not required for the nutrition of this organism ("W" strain) and that the decreases in yield observed when a sucrose purified by means of alcohol (with the object of removing "bios" and "co-enzyme R") is employed are almost entirely due to the removal of zinc and molybdenum. The beneficial effect of yeast, malt extract, etc., usually ascribed to accessory substances, may also be due to heavy metals thus introduced into the medium.

Metabolic Products

Formic acid is stated by Takodoro (1935) to be a product of the fermentation of arabinose by *Aspergillus oryzae*. Other products include citric and glycollic acids with smaller amounts of oxalic and kojic acids and *glyceraldehyde*. From fucose formic, glycollic and lactic acids are produced.

Terrestrial acid. This acid, isolated from the metabolic products of *Penicillium terrestre* Jensen by Birkinshaw & Raistrick (1936), is closely related to the series of substituted tetronic acids produced by *P. Charlesii*. It is actually an ethylcarolic acid, the hydrate having the constitution α -(l - γ -hydroxy- n -hexanoyl)- l - γ -methyltetronic acid.

Attention has been drawn to the similarity in constitution of the *P. Charlesii* acids to vitamin C (ascorbic acid). Bernhauer *et al.* (1936) have now shown that *Aspergillus niger* on various substrates produces a reducing substance which is similar to ascorbic acid since, like that substance, it can be titrated with dichlorophenol-indophenol in acid solution. This is usually regarded as a fairly specific test for ascorbic acid. The conditions for production of the best yield have been studied, but the reducing substance has not actually been isolated so that its identity is not yet established.

Mycelial constituents. *Oospora sulphurea-ochracea*, when grown on koji wort, produces three crystalline compounds, viz.: $C_{17}H_{16}O_8$, needles, M.P. 214° , giving a violet colour with $FeCl_3$ in alcohol; $C_{10}H_{10}O_4$, pale yellow needles, M.P. 257° (decomp.), giving a green colour with $FeCl_3$ in alcohol; $C_{17}H_{16}O_8$, rhombs, M.P. 200° (decomp.), giving no colour with $FeCl_3$. A fourth compound, $C_{18}H_{18}O_8$ (?), M.P. 190° , is sometimes obtained (Nishikawa, 1936).

By extraction of the dried defatted mycelium of *Aspergillus sydowi* with acetone Woolley & Peterson (1936) obtained the amino acids leucine and isoleucine.

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POPULATION PROBLEMS OF SOCIAL INSECTS

By F. S. BODENHEIMER

(Hebrew University, Jerusalem)

(Received 17 May 1936)

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THE population problems of social insects are twofold. We have to distinguish between the growth of the average or individual colony of any species, i.e. the change of its populations with age, which is often mixed with seasonal fluctuations, and the annual or secular fluctuations in the number of colonies of any species in a given area.

The fluctuations in the number of colonies depend mainly on such environmental factors as suitable conditions of breeding, food, and suitable climatic conditions during the most sensitive stages of colony development. We do not intend to deal here with this aspect of the population problem of social insects, but to restrict ourselves almost entirely to the problems connected with the development of the individual colonies. Quantitative population studies of animals, especially of social insects, have been neglected, as, indeed, on most other animals, in spite of the fact that the social insects have attracted such wide interest in many other respects. This article has been written in the hope that by pointing out the wide gaps in our knowledge of the dynamics of the populations of social insects we might stimulate future studies to fill these gaps.

I. TERMITES

(1) *General biology of termite colonies*

The termites are primitive insects, which though living in colonies have no affinity to the highly developed social Hymenoptera. They abound in the humid and semi-humid tropics, but extend also to sub-tropical climates, where they even penetrate into deserts (Snyder, 1915; Hegh, 1922).

Every colony contains one, or, less frequently, several royal pairs (sexuals). The workers as well as the soldiers are of both male and female origin. At the death or absence of the primary sexual forms, supplementary reproductives may be bred within a few weeks, from fifth or sixth, rarely from seventh instar larvae. Colonies which have segregated from a mother colony may receive their sexual forms either in the way just described (supplementary sexuals) or by adopting strange primary sexuals (*Eutermes amboinensis* Weyer, 1930).

(2) *Quantitative data on termite populations*

The populations of fully grown termite colonies show a wide range: from several hundreds (*Kaloterms*, *Leucoterms*) to some millions (in some of the tropical species, which build large hills). Emerson (Kofoid *et al.* 1934) counted 3,000,000 inhabitants in one colony of the South American *Nasutitermes surinamensis*; the African *Termes bellicosus* is certainly no less populous and the same is probably true of the Celanese *T. redemanni* and others. A population of the Australian *Eutermes exitiosus* was 1,806,500 in a not yet full-grown hill, and approximately 44,000 alates (2.4 per cent) would have emerged from the mound if swarming had occurred (Holdaway *et al.* 1935). A colony of a species at Jamaica contained 631,878 individuals, nine-tenths of which were fully grown workers (Andrews, 1911).

All these giant nests are those of subterranean species. The colonies of the wood-dwelling species are much smaller, as will be seen later. But some of the carton-nest building tropical species are very populous. Thus Escherich (1911) estimates a provisioning procession of the Celanese *E. monoceros* at about 200,000 individuals, although naturally this number includes only a part of the total nest population.

The egg production of the queen fluctuates not only from species to species, but also with age. Every queen, when founding a colony, is still relatively sterile (15–50 eggs in the first season). But, under proper care of the worker, her abdomen continues to grow, until, in some tropical species, the rest of the body is only a small appendix. This abdomen is filled mainly with the ovaries; Bugnion (1914) counted in the abdomen of one queen not less than 48,000 eggs in different stages of maturation. The high egg production in the large tropical nests can be seen from the fact that Escherich (1911) counted in one of the many fungus gardens, existing in every nest, not less than 300,000–350,000 eggs. Fuller (1915) observed a laying rate of 4000 eggs per day in an African termite. Emerson (Kofoed *et al.* 1934) counted 7000 eggs per day in the South American *Anoplotermes silvestrii*. Escherich (1909) observed in four different queens of *Termes bellicosus*, from hills of various sizes in East Africa, an oviposition rate of one egg every 2 sec.; this would make the daily number 43,000. But it cannot be assumed that egg laying must continue at this rate. While in tropical species oviposition continues uninterruptedly throughout the year it is interrupted in subtropical regions during the cold season. The range of possible oviposition rate in termite queens of different species and ages is from 43,200 eggs to 1/20 egg per day (15,400,000 to 18 eggs per annum).

(3) Potential immortality and foundation of the colony

The termite colony possesses a potential immortality due to the appearance of supplementary reproductives. But Kalshoven (1930) has stated definitely that there is a limited longevity for the colonies of the wood-termite *Kaloterms tectoniae*. In dry-wood species the appearance of supplementary sexuals is less common than in species living in damp wood or subterraneously. It is certain that Californian colonies show higher average longevity than those of *K. tectoniae*. But only on very exceptional occasions have colonies older than 10 years been studied (Kofoed *et al.* 1934).

The normal foundation of a primary colony begins with the nuptial flight of the alate sexuals. After coming back to the soil, the male follows the smell trail of a female, the wings are shed and the royal pair begins to excavate the royal cell in the soil or in wood. There it lives for some time together, and only then does the first copulation occur. During the first period of the foundation of the colony the royal pair has to perform all the work, including the nursing of the progeny. This nursing is not very carefully done during this period; part of the eggs perish and there are certain indications that they are occasionally devoured by their parents. Only when the first workers begin to take care of the feeding of the queen and of the progeny is the effective nursing of the latter guaranteed, and the increasing growth of the queen runs parallel with a corresponding increase in oviposition rate. The male lives as long as the female and copulation is frequently repeated throughout life.

(4) *Description of Kaloterms colonies*

We are fairly well informed on the growth of the colonies of the genus *Kaloterms*. The classical study of Grassi & Sandias (1897) is devoted to *K. flavicollis* F. in Italy. The colonies in this species are already considered populous when they contain about 500 members, and they rarely surpass 1000 individuals. Fifteen months after the nuptial flight the royal pair may be surrounded by 15–20 young ones, after another year by about 50 individuals. In the 2 or 3 following years the royal pair grows to its full size and the population increases until it reaches a maximum, after which it becomes nearly stationary for some years.

After 2 years 8–10 alate sexuals may depart from the nest and in successive years the number leaving increases, parallel to the increase of the population. The queen reaches maximum fertility at an age of 3–4 years (4–6 eggs per day). A supplementary reproductive is grown for every primary sexual that dies. Soldiers are relatively scarce (2–4 in nests of 8–15 inhabitants; in larger colonies 1 soldier to 15–20 workers). Development is arrested in winter (mid-November to mid-April). The nest contains about 100 eggs in the winter, which begin to hatch from early June to the end of July (5–10 per day). Oviposition is restricted to the periods between end of May and end of June, and to September–October. Swarming of alate sexuals occurs from July to October. The shortest development is about 13 months.

We possess a good series of quantitative data on the growth and dying out of colonies of the Malayan *Kaloterms tectoniae* Damm. (Kalshoven, 1930). This termite develops in the branches of trees. The royal pair may live throughout the lifetime of the colony, but when it is replaced by neotenic forms, the lifetime of the colony is not considerably prolonged. The oviposition rate of the queen is very low at the beginning and only a part of the eggs laid develop successfully. The oviposition rate of the queen increases with age up to 2–3, or even 8, eggs per day. The egg stage lasts about 56 days. The decline which speedily follows upon the population maximum is due to a decrease in egg production and to losses of winged sexual individuals by swarming (October–December). The winged sexuals are not normally produced before the sixth year. The drying out of the wood of their host plant increases swarming tendencies.

Fig. 1 shows three types of life histories of *Kaloterms* colonies. The small and slowly growing colonies reach their population maximum at the age of about $4\frac{1}{2}$ years and die at an age of about 6–7 years. Most colonies reach the maximum after $5\frac{1}{2}$ years and die at an age of about 10 years. The larger colonies reach their maximum only after a period of 8 years and die at an age of 15–16 years. The average maximum of these three groups is 230, 840, 2850 individuals respectively.

The most important conclusion to be drawn is that the vitality of the colony is determined either by internal, or by external factors, at the moment of its foundation. While the final relation of the population maxima of the three groups is 1:3.6:12.4, we find a relation of 1:6.6:17.6 at the end of the first year.

It is certain, therefore, that in the case of *Kaloterms tectoniae* it is not the pro-

gressive increase in inhabitants with age, but *primary* differences which determine the future development of the colony from the beginning.

The growth of the colony is well expressed by logistic curves. The equations for the three different types of life histories are inserted in Fig. 1.

At the end of the second year 30–220 eggs are present. If all of them develop successfully, 40–230 nymphs would be present two months later, whereas in reality only 20–120 individuals are present (similarly at the end the first year 10–30 individuals for 20–60 eggs). Eggs may die because of insufficient care or, what

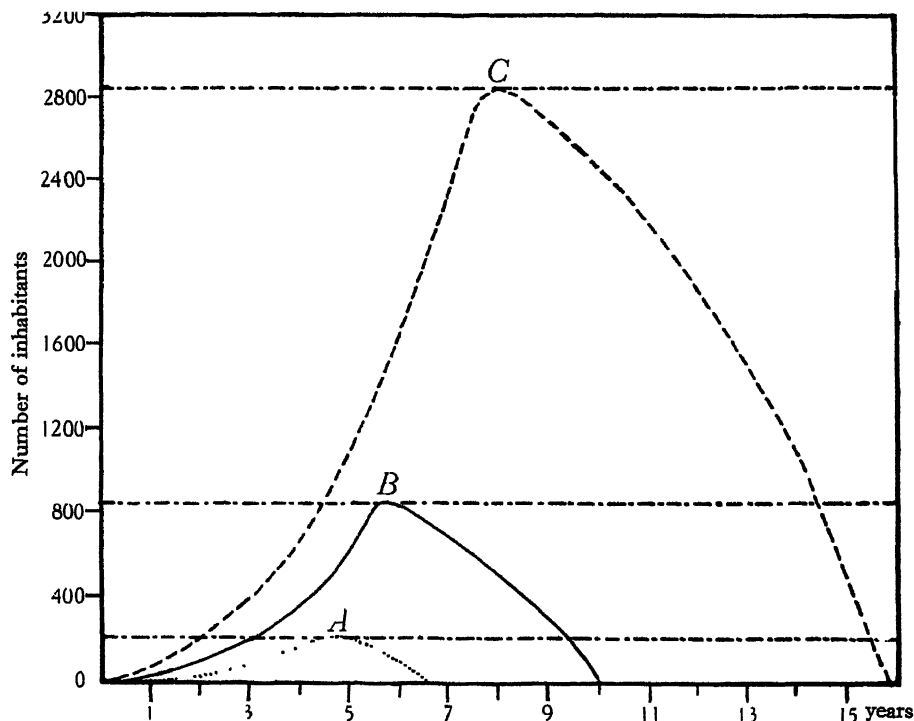


Fig. 1. The growth curve (logistic curve) of *Kaloterms tectoriae* colonies (adapted from Kalshoven). A, small; B, medium; C, large colonies.

$$A, y = \frac{240}{1 + e^{5.5130 - 1.6628x}}; B, y = \frac{844}{1 + e^{4.16137 - 0.95962x}}; C, y = \frac{2844}{1 + e^{3.31284 - 0.87363x}}.$$

more frequently occurs, they may be eaten by the parents. The diminution in the number of eggs, which has also been observed by Weyer (1930) in other species, is no longer observed in later stages of the colony development.

The Californian *Kaloterms minor* Hgn. has been studied in recent years (Kofoid *et al.* 1934). The alate sexuals swarm in October. The excavation of the royal cell is followed by a period of inactivity (often of 9 months' duration, which many pairs do not survive). Two years after the colonization pair has entered the wood, the colony will have destroyed about 3 c.c. of wood and will be securely established (royal pair, 1 soldier, about 12 workers). Oviposition lasts from late spring to late autumn. 1–2 eggs per day are laid during 7–10 days; oviposition ceases thereafter

for a month or more and is then resumed at the same rate. In the second year the primary queen lays about 8 (max. 15) eggs. Fecundity increases with each succeeding year, probably reaching the maximum at an age of 10–12 years. It then drops, perhaps rapidly, with secondary queens appearing in the colony to assume the functions of the primary queen. The progeny of the primary queen, produced intermittently from spring to autumn, represents the annual increment of the colony. Development into the fifth instar lasts from 131 to 180 days, into the seventh instar from 188 to 415 days. The entrance of the colonizing royal pair does not yet mean successful establishment. Among 137 colonies only 5, i.e. 3.6 per cent, survived the first year (Kofoid *et al.* 1934).

The growth of colonies of *K. minor*, as well as that of the subterranean *Reticulitermes hesperus* (3000–100,000 inhabitants) and of *Zootermopsis angusticollis*, a species living in the damp wood of rotting stumps (4000 inhabitants), have been described by Kofoid *et al.* (1934).

(5) Conclusions

The dangers awaiting the swarming alate sexuals upon leaving the parental nest are manifold: predaceous insects, birds, lizards, mammals, etc., all feed on them. Very many are destroyed by unfavourable climatic conditions, by lack of suitable breeding localities, etc. And the relatively few pairs which successfully enter the soil or wood are by no means definitely established. Only a small fraction of these pairs survives the first or second year. They die mainly because of unfavourable environmental conditions (climate, etc.). Even during the slow increase of the initial period they are still exposed to attacks by enemies. Kalshoven (1930) has observed that colonies of *Kaloterms tectoniae* are liable to destruction by ants (such as *Tetramorium*) until there are 50–75 inhabitants.

The long life of the inhabitants is an outstanding feature. In some cases they are known to have lived at from 3 to 5 years, whereas the queen may live much longer. Additional data on the longevity of termites of all castes are still required. The development of the colony largely depends on external factors, especially on food supply, temperature, humidity and on proper care given to the queen and the offspring. Apart from environmental conditions the size of the colony depends on age, and on seasonal fluctuations especially in young or small colonies (including loss of swarming alates). Only very few of the numerous alates succeed in successfully establishing new primary colonies. They have been aptly compared to the seeds of a tree. However, many species form new colonies occasionally or, as a rule, by segregating some hundreds or thousands of workers from a maternal colony and producing their own sexuals either by breeding neoténics from their own larvae, or by adopting stranger primary alate sexuals.

There is no proof of the immortality of termite colonies and the facts rather tend to show that these colonies have a definite longevity. Further observations on this point are very much needed.

II. ANTS

(1) General biology of ant colonies and their foundation

Despite the lack of affinity between termites and ants, the development of their colonies has many features in common. Ants abound in the tropics, and are also rather common in temperate climates.

Ant colonies are, as a rule, monogynous; even if some queens seem to co-operate during the first period of colony foundation, they fight each other to the death as soon as the first workers appear. Fertilized females may be favourably received only in very large nests with segregating colonies like those of *Formica rufa* or *F. exsecta*. But this polygyny or pleometrosis is often only a temporary state until the definite segregation of the daughter from the mother colony has taken place. However, in other colonies of *Formica*, *F. exsectoides* for example, as many as 10–30 queens have been found in one nest before hibernation (Andrews, 1929). The workers and soldiers are all of female sex. The colonies are founded primarily by fertilized queens. Fertilization occurs only once during the nuptial flight after which the males are no longer needed. As a rule, they die soon after the nuptial flight. The queen builds a cell in soil or wood and sooner or later begins to oviposit. She has to take care of the young brood until the first workers hatch. These first workers are nanic, as is the case with the termites. The number of the workers, as well as the fertility of the queen, only rises when the queen is properly fed and sufficient workers are present to nurse.

Most ant colonies depend upon the life of their queen. Supplementary sexuals are not produced, nor are strange fertilized queens adopted by most species after the death of the queen. But in certain species (especially of *Formica*) such adoption, probably of fertilized daughter-queens of the same nest, occurs regularly. Old nests (nests up to 80 years old have been observed) must have been maintained in this way (Bischoff, 1927).

Apart from this independent mode of colony foundation, various kinds of ants are known in which the females have more or less lost the faculty of independent colonization. Many species of *Formica* from Europe and North America (*F. rufa*, *F. exsecta*, *F. truncorum*, *F. exsectoides*, etc.) are adopted by nests of *F. fusca*. With the death of the *F. fusca* queen, no new workers of this species are produced and the colony slowly grows into a pure nest of the adopted species. We find similar adoptions of *Lasius mixtus* or *L. umbratus* by the nests of *L. niger*.

Santschi observed that the female of *Bothriomyrmex decapitans* enters the nest of *Tapinoma nigerrimum* and decapitates its queen. Even if the major part of the *Tapinoma* colony leaves the nest, sufficient workers remain to help the *Bothriomyrmex* queen to nurse her brood (Bischoff, 1927).

The history of a *Formica truncicola* colony adopted by *F. fusca* has been studied by Wasmann (1905). Wasmann estimated the maximal longevity of a colony to be 20 years, because of the known fact that a *Formica* queen may survive 12 years and new queens may subsequently be adopted by the colony.

These nests are mixed only temporarily and finally become a pure nest of the

"adopted" species. In other cases, nests may be permanently mixed in species which only maintain their existence through doulosis (robbing pupae of other species) or by the alliance of queens of different species.

(2) *Quantitative data on ant populations*

Actual counts of ant populations are rather scarce. Quantitative data may be found in the papers of Bellevoye (1891), Donisthorpe (1915), Forel (1920), Skwarra (1928), Goesswald (1932), Eidmann (1927), Hoelldobler (1928), Hubrich (1929), etc. Very many species contain only from about 12 up to 100-300 workers. However, we lack quantitative evidence concerning the most populous colonies, as those of *Tetramorium caespitum*, the leaf-cutting *Atta sexdens*, the large robber species like *Eciton* and *Dorylus*. The only available data for the populous and abundant group of harvester ants are on one nest of the North American *Pogonomyrmex barbatus* F. A nest of average size (7 ft. in diameter, 15 ft. deep) contained 436 chambers and 12,358 ants (Wildermuth & Davis, 1931).

Pricer (1908) states that for *Camponotus pennsylvanicus* adult colonies contain from 1943 to 2500 workers and that they must be from 3-6 years old before they are able to produce the sexual phases.

The large and populous hills of *Formica* have often attracted the attention even of laymen. The most competent authorities have estimated that the large hills of *F. pratensis* contained from 100,000 to 500,000 individuals (Forel, 1874, based on observations on migrations). Escherich (1917) accepted an average of 150,000-200,000 ants for the larger hills.

The most comprehensive census has been made in Switzerland by Young (1900) for *Formica rufa*. From one hill (60 cm. high, 114 cm. wide), after treatment with carbon disulphide, he excavated 22,580 adults and 13,500 development stages. Not all adults were caught, so that a few thousand individuals must be added to the actual count. Young carefully collected the active adult population of 5 other nests (ranging from 45 to 70 cm. in height and from 95 to 160 cm. in diameter) by various other methods. The census of adult working inhabitants was as follows: 19,933, 47,828, 53,018, 67,470, and 93,694 ants. Since some ants always escaped the census, an addition up to 10,000 individuals per nest should be made. The large range of adult inhabitants (about 20,000-100,000) showed no correlation with the size of the nests. The largest mounds were by no means the most populous. Young's hope that these studies would be continued has scarcely been realized.

The only other count known to the writer is a census of an average ant hill of *Formica exsectoides* F. (19 in. high, 15 ft. in circumference) in Maryland by Andrews (1929). The ants were taken in semi-torpor in February, and again in May. The total adult population was 7124 workers (living), 1104 workers (dead), and 11 queens, altogether 8239 individuals, which had entered hibernation the preceding winter. Taking into consideration all possible gaps in this census, the total adult population of this mound certainly did not exceed 10,000 individuals.

From all these counts it may be concluded that the high estimates of Lubbock and Forel are certainly exaggerated. The normal adult population of the large hills

of the different *Formica* species range between 5000 (small colonies of the less populous species) up to 100,000 (approximate maximum of the most populous species). Facts on the numerical relation of developmental stages to adult population in the different seasons are still completely lacking, so that nothing can be written on the dynamics of ant populations. Andrews (1929) assumes for *F. exsectoides* a breeding season of 3 months with 3 broods. The 11 queens should rear 1320 adults per month, so that the yearly total addition would amount to 3960 adults (not allowing for the mortality of these individuals and of the previously existing population). But these are only assumptions.

Formica multiplies not only by primary sexuals, but also very commonly by segregating daughter colonies. The daughter colonies may remain connected with the parent colonies or they may be strictly separated later on, so that one colony may colonize quite a considerable area with such daughter colonies. Fifty such colonies have been observed for *F. rufa* (Wasmann, 1909), 200 for *F. exsecta* (Forel, 1874) and 1600 for *F. exsectoides* (McCook, 1883). In this way, the production of one original mother colony may extend into many millions. These daughter colonies, however, should be regarded as distinct colonies the moment they segregate from the mother colony. This type of reproduction is comparable to the asexual reproduction of lower animals by division, whereas the primary colony foundation by a fertilized female corresponds to the sexual reproduction.

(3) *The early history of ant colonies*

The nuptial flight is generally restricted to a short season. The males, as a rule, exceed the females considerably in number, whereby the fertilization of every queen seems best guaranteed. The dangers awaiting the fertilized queens, which shed the wings immediately after the nuptial flight, are not less than those for termites. In ants, also, the first stages of primary colonization are the most sensitive stages of the development of the colony. Wildermuth & Davis (1931) report on the enormous number of queen cells of *Pogonomyrmex* and Eidmann (1935) of *Atta*, the overwhelming majority of which perish in a very early stage.

The queen, which has to do all the work, including nursing the brood, until the first workers appear, generally does not partake of any food from outside for a period of about 100 days to over 1 year. But she devours part of the eggs or young larvae and also feeds them to the first larvae. Viehmeyer (*vide* Escherich, 1917) reports that from several thousand eggs of *Formica sanguinea* only about 20 developed successfully. From 903 eggs of *F. ulkei* only 1 reached the pupal stage (Holmquist, 1928a). Lubbock (1892) reports on eggs devoured during the colony foundation of *Myrmica ruginodis*. In *Messor structor* some 10–30 out of 48–81 eggs were devoured before the appearance of the first workers. Swarming takes place at the beginning of April. Some queens produced up to the beginning of winter 6–20 workers and 0–13 larvae, which hibernated. In another year from 22–45 workers were reared up to the end of the summer (Meyer, 1927). One queen of *Formica fusca* laid at least 9 eggs, from which only 1 worker developed (Eidmann, 1929), whereas in *Camponotus herculeanus*, at the end of the experiment, from at

least 14 eggs, 1 developed into a worker, 2 into pupae, 9 into larvae (Eidmann, 1926).

The colony foundation of the corn-field ant *Lasius niger americanus* Em. has been studied very thoroughly by Tanquary (1913). The nuptial flights of this species occur in August–September. Either no eggs are laid before hibernation, or if laid they perish. Oviposition begins the following spring. The total egg production in 3 colonies was 325, 530, 171 (342 on the average), whereas the number of workers produced was 11, 27, 11 (16.3 on the average). The queen eats a large proportion of the eggs and probably uses them in feeding the first larvae. Only 4.8 per cent of the total egg production of the first year developed into adult workers.

The total development (egg to worker) lasts 85–98 days, or when hibernating even longer than 1 year. No sexuals are produced in the first year. After the second year the average sized and the large colonies increase rapidly as the number of workers by that time is large enough to provide plenty of nourishment for the queen to lay a much larger number of eggs. The fertility of the queen rises with better feeding and with the increasing number of workers inhabiting the nest. Queens of large nests laid in two cases 341 eggs within 24 hours and 162 eggs within 19 hours.

By the beginning of the third year the average colony is so large that it can afford a high fertility of the queen, proper nursing for the increased offspring and even the production of alate forms. But the small colonies with only 2 workers at the end of their first year will only grow as populous as the larger colonies of the same age a year or two later. And these nanic colonies may produce alate sexuals only in their fourth or fifth year.

In *Atta* the queen devours up to 90 per cent of the eggs laid, whereas the first workers of this tropical ant appear already during the second month after the foundation of the colony. The queen has to feed the first larvae with eggs and to prepare the fungus gardens for larvae which are bred later on. The queen lays about 50 eggs daily during the first period of the colony foundation. About 2000 eggs are deposited before the first workers appear, but the number of developmental stages (eggs to pupae) never surpassed 120–150 in the observations during this period. The beginning of the colony foundation is rather quick in this tropical species. Nests observed 10 days after the nuptial flight contained 50–60 eggs, 3½ months after the nuptial flight 160 workers, 150 larvae and pupae and 50 eggs (Huber, 1905).

In one case only do we possess the full history of one colony. This is a colony of *Lasius alienus* in an artificial nest (Janet, 1904). Conditions must have been rather unfavourable, as the size of the colony remained small as compared with those observed in nature. The queen lived for 9 years and 3 months. The underlying principle of the development of this colony is analogous to that of termites (Fig. 2).

(4) *Fertility and longevity of ants*

Data on the fertility of the queens are extremely scarce. The queen of the small colonies of *Ponera* lay about 2 eggs in one clutch and then pause for several days or

weeks. During the main oviposition period of *Formica sanguinea* (Escherich, 1917), the interval between the ovipositions was 10 min. on the average, but rose to 1-1½ hours later on. A fully grown queen of *F. exsectoides* began to oviposit at a rate of 4 eggs per day (oviposition period 3 months), whereas those of *F. ulkei* (oviposition period 1 month) produced 3 eggs per day throughout the whole period (Andrews, 1929; Holmquist, 1928a; additional data on *Lasius americanus*, Tanquary, 1913).

Another point on which we urgently need information is the longevity of the ants. There is much scattered information on the duration of development (egg to adult) for many species. But as this depends primarily on the environmental temperature and most breeding was done in the laboratory at temperatures different

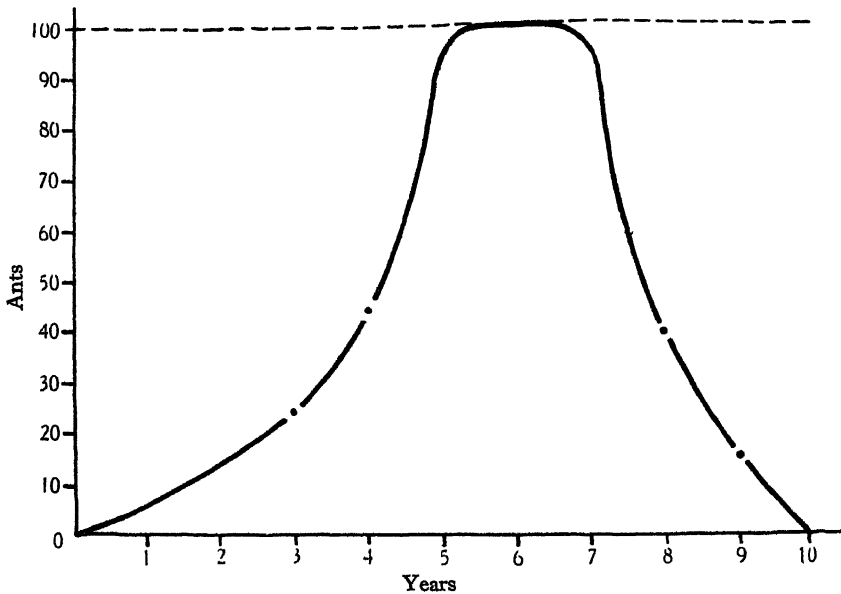


Fig. 2. The growth curve (logistic curve) of a small colony of *Lasius alienus* (computed after Janet).

$$y = \frac{100}{1 + e^{4.4204 - 0.3029x}}$$

from the normal conditions of breeding in nature, they help us relatively little. As far as the longevity of the queen is concerned, it certainly lasts for some years, in some cases up to 10 and even 15 years (*Formica* and *Lasius*; Doflein, 1914). In some workers, also, longevity up to 5-6 years has been maintained. Observations made under the unnatural conditions of artificial nests help us very little. One has to distinguish, furthermore, between the physiological and the natural death of the workers and soldiers, i.e. from senescence, or accident. Premature death by enemies, unfavourable situation during hibernation, etc., may be considerable. Goetsch (1929) has observed in *Messor* that the recently hatched individuals in the hill serve as nurses and house-ants (analogous to house-bees) and later, after passing through a stage as guards, change to field work.

(5) *Conclusions*

The dangers which accompany primary colony foundation by queens are of no less importance than those of termites. The most populous species propagate quicker, and with a much higher factor of safety, by segregating daughter colonies and adopting queens. These large colonies are often secondarily polygynous, whereas small colonies are always monogynous. More complicated systems of colony foundation have been developed in many species (dependent colony foundation). The principle is that workers of other species—at least during the sensitive period of colony foundation—are used for nursing the brood. In the independent colony foundation, the queen, which starves until the first workers are reared, utilizes part of the eggs for her own nutrition (apart from the histolysis of the wing musculature) and for that of the first larvae.

Queen and workers seem to be rather long lived (several years) as a rule, but exact observations are badly needed. Nothing is known of the growth of the colony beyond the first year of its existence and the data on the final size of the colonies are still extremely scarce.

The age of the colonies depends, in those strictly monogynous species which refuse adoption, on the longevity of the queen. In cases where adoption occurs, the colony may far outlive the foundress queen. However, there are no indications of actual immortality. It seems that in most populous nests, which are also the long lived, environmental conditions change unfavourably with age and finally induce the dying out of the colony, although it may still survive in segregated daughter colonies.

Nothing is yet known of the dynamics of colony development in ants, beyond the first year, and ant populations are in every respect much less known than those of termites.

III. SOCIAL WASPS

(1) *General biology of wasp colonies*

The normal life history of social wasps is as follows. In spring, generally in late spring, the fertilized queens, which have successfully hibernated, begin to build a nest of a few cells, in which they oviposit. The first workers, leaving these cells, enlarge the nest and take proper care of the brood. Oviposition continues and at the end of the summer, as a rule, the first large queen cells appear. Finally unfertilized eggs are laid, from which the drones develop. The new sexuals copulate; the organization of the population disintegrates even before the approach of the bad season, the old queen, the workers and the males die, the larvae which are still present perish, and the young, fertilized queens disperse in search of suitable refuges for hibernation.

The direct observations on population growth of wasps (with the exception of *Polistes*) are attended by so many difficulties, that up to the present hardly anything beyond the study of the nests in the different stages of development has been

made. Many of the problems connected with the population growth of social wasps have recently been discussed by Betz (1932).

The number of cells per nest is not identical with the number of individuals reared. The same cell is often used in the same season for a second or even for a third brood. While in the species with a few cells only (*Polistes*) every cell normally serves for breeding at least one individual, in the very large nests of social wasps a few cells may remain empty. The number of cells, however, permits a rough estimate of the total breeding activity of the nest during the season, as will be shown later.

Another gap in our knowledge concerns the longevity of the workers. It is certainly limited (Ritchie, 1915, 3-4 weeks; Janet, 1903, 1-6 weeks). New workers must always be bred to maintain the population. But more exact evidence on this point is needed, and the modern methods of marking should solve this question.

Finally, we are insufficiently acquainted with the length of development of the different stages and castes at the normal nest temperatures. They depend in part on nutrition. The first larvae, attended by the queen, grow much more slowly than the later ones, which are more adequately nourished by the workers (Siebold, 1871). It is hopeless to attempt a reconstruction of the seasonal dynamics of a wasp population before knowing a great deal more about all these points.

(2) Quantitative data on wasp nests

We now turn our attention to some of the available quantitative data. The number of combs ranges from 1 to 15, that of cells from 32 to 11,900 (count by Fabre; 16,000 estimate by Réaumur, 20,000 estimate by Rouget; quoted from Betz, 1932). We quote some counts of the seasonal fluctuations in the numbers of adults for 4 species. It is well understood for the above-mentioned reasons that these figures do not yield information on the real dynamics of the seasonal development.

The normal maximum for adult populations of the common wasp (*Vespa vulgaris*) is estimated to about 2640 individuals. But Crawshay (1905) reports on a giant nest of this species, which contained adults and 6389 eggs, larvae or pupae. The extraordinary size of this nest is due to especially favourable environmental conditions.

With regard to data on *Vespa diabolica* and *V. maculata*, etc., the reader is referred to Baerg (1921), Rau (1929), Schmidt (1917) and Betz (1932).

Two different stages in the development of the colonies of true wasps have recently been analysed by Weyrauch (1935). The genus *Vespa* may be subdivided into the more primitive subgenus *Dolichovespula* (1200 adults, 500 cells) and the more socially integrated subgenus *Vespa* (1000 adults, 5500 cells).

In *Polistes gallica*, Steiner (1930a) observed from 1 to 19 adults throughout the season.

The classical observations of Siebold (1871) confirm the assumption that during late summer the number of adults does not surpass 26 (maximum). The number of cells in the single comb fluctuates under normal conditions between 50 and 120 (30 and 230 being extremes).

Fig. 4 illustrates in a preliminary way the growth of the adult population in 3 species of *Vespa*, which gives a logistic curve. The disintegration speedily follows the population maximum. Additional data and very instructive figures are contained in Janet (1903).

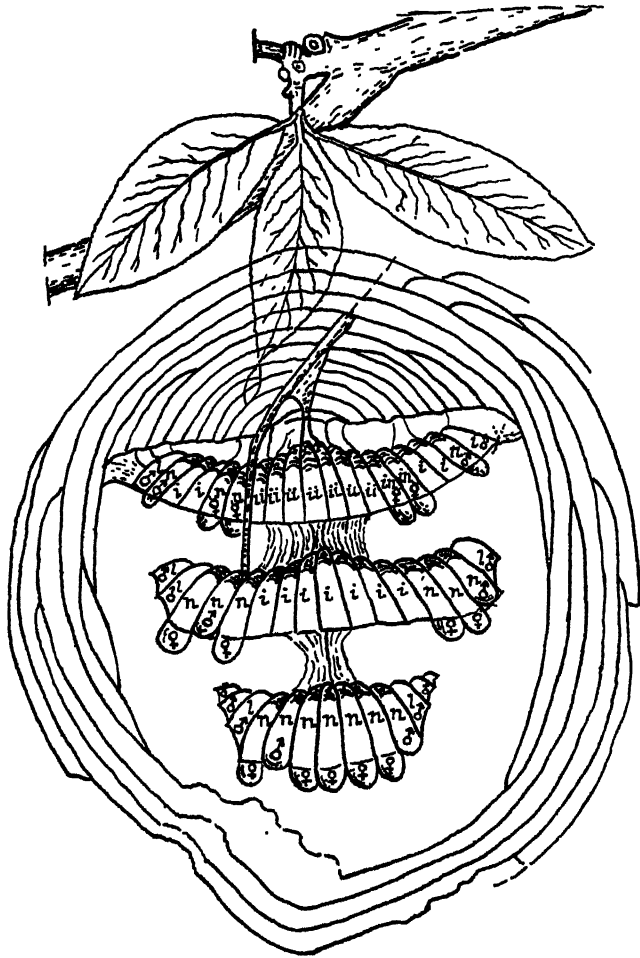


Fig. 3. Nest of *Vespa media*. *i*, cell from which one adult hatched; *ii*, cell from which two adults hatched; *n*, cell with nymph (after Janet).

Little is known of the populations of the large nests of Brazilian wasps. Ihering (1896) counted 3074 adults (including 34 females) in one nest of *Polybia scutellaris*. The colonies of this genus, as well as those of *Chatergus*, *Nectarinia*, and others, do not perish in tropical South America during the winter but persist throughout the year. Other social wasps (*Polistes*, *Pseudopolybia*, and others) live in the same way as just described for Europe and North America. Dr C. E. Pemberton of Honolulu was kind enough to inform the writer that *Polistes macaensis* and *P. olivaceus*, two oriental wasps introduced into Hawaii, gather in clusters during the

autumn and winter months under the eaves of houses, among vegetation, etc., and remain clustered there until the following spring.

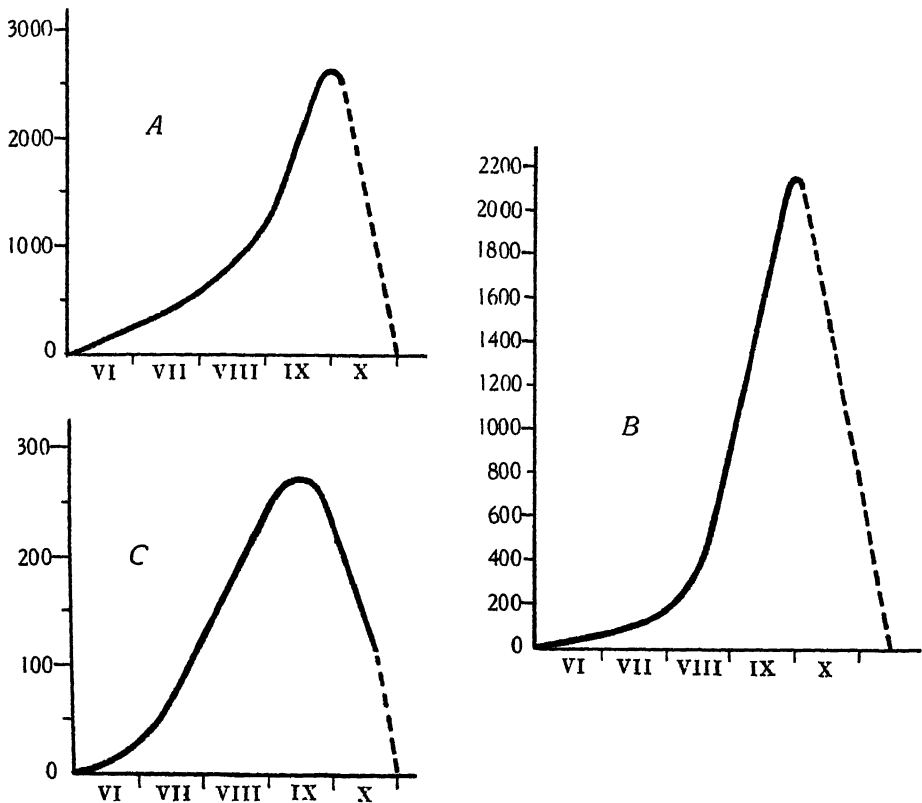


Fig. 4. The growth curve in some wasp colonies. A, colony of *Vespa vulgaris*; B, colony of *V. diabolica*; C, colony of *V. maculata*. The ordinate indicates the number of individuals in every stage, the abscissa the date.

(3) *Dynamic analysis of the seasonal population trend in a nest of Vespa crabro*

A classical paper of Janet (1895), which seems to be almost forgotten, gives a very exact description of a nest of the common hornet (*Vespa crabro*) during 1894 at Beauvais. The first 74 days of the nest are described in detail and sufficient indications are given for computing the remaining history up to the dispersion of the nest in November.

The data on which the calculation was based are as follows: (1) The actual number of eggs laid from 15 May until 27 July. On 30 July 12 eggs were laid in 1 day. In another nest during the summer an average of 9–10 eggs were laid for several days. It was therefore assumed that until the end of August 8–9 eggs had been added daily with the exception of a depression in early August, when building on the combs was greatly reduced. This depression was very probably accompanied by a reduced rate in oviposition.

(2) The average actual duration of the different stages was for those individuals which started development in the respective months, as follows:

Oviposition	Egg	Larva	Pupa	Total development days
May	18	18	15	51
June	12	16	14	42
July	5	12	13	30
August	5	12	13	30
September	12	16	14	42

The data from May to August are based on the observations of Janet. Those for September have been assumed, but, as the comparison with the history of the nest shows, the actual development was much more retarded.

(3) The observed longevity of 9 workers was: 7, 15, 6, 27, 2, 21, >41, >32, >25 days. Adding 5 days to each of the last three values, we receive an average value of 21 days. In the calculation the adult longevity was alternatively estimated to be 14 or 21 days. In nature the longevity of the first and last born adults is probably shorter, that of the individuals born in the summer relatively longer. The building of a second comb began on 24 July, of a third comb on 21 September. On 28 September the first 2 males left the nest in which they had hatched, probably several days earlier. The queen died on 1 October. The last worker died on 14 November, at which time 54 large larvae, deriving from the last ovipositions of the queen, were still in the nest. The low temperature of the disorganizing nest is the reason for this excessive delay. Under natural conditions they should have died before winter.

Fig. 5 shows the calculated population for every week of this nest of *V. crabro*, when the workers' longevity is assumed to be 3 weeks.

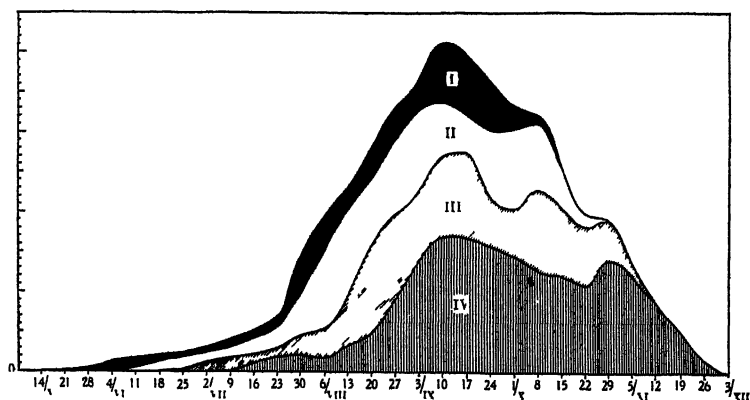


Fig. 5. The population trend of a nest of *Vespa crabro*. I, eggs; II, larvae; III, nymphs; IV, adults present in a colony. Computed after observations from Janet. The ordinate indicates the number of individuals in every stage, the abscissa the date.

The agreement of the calculated with the observed values is very good. The basic fact, which is very well illustrated by Fig. 5, is that the early death of the

queen is the beginning of the disorganization of the colony. Desertion of the nest, insufficient nursing or even killing of the brood, etc., hasten it. This explains why wasp colonies die *before* the bad season actually forces the disintegration. This theory finds its confirmation in the history of a nest observed by Mueller (1818).

The total egg production of Janet's nest was as follows:

Month	Days	Eggs	Eggs per day
May	15	7	0.5
June	30	24	0.8
July	31	126	4.1
August	31	245	7.9
September	30	168	5.6
Total	137	570	4.2

The nest was a rather small one. It had only two large combs and a small third one.

570 individuals have been bred in 240 cells, which is explained in the following way:

Comb I:	114 cells, 3 breedings per cell =	342
Comb II:	102 cells, 2 breedings per cell =	204
Comb III:	24 cells, 1 breeding per cell =	24
Total		570

The repeated breeding in one and the same cell has been proved for several species of wasps by Janet (1895, 1903) and is probably a very general fact. Another interesting fact discovered by the same author is that only young workers are really active in building additional cells, whereas older workers may participate in the building of the outside covering of the nest. Further signs of division of labour could not be ascertained. Any temporary lack of young workers is accompanied by reduced building activity.

(4) *The number of cells in relation to the total annual production of the wasp colony*

This estimate of the number of broods per cell should be compared with the observations of Ritchie (1915). In one nest of *Vespa vulgaris* he found 6 combs with 4217 cells in July, i.e. at the beginning of the large increase of the population. 417 workers were living, whereas 852 workers had hatched during the season. This fact in itself demonstrates the limited longevity of the workers. In this early stage of development 680 cells showed signs of a second, 172 of a third brood. This means that every cell from which a pupa is hatched is, under normal conditions and as long as the nest is still greatly increasing, immediately filled with a new egg, which confirms the observations of Janet (1903). The total production of the following nests should therefore be estimated as follows:

Species	Nest	Month	No. of cells	No. of adults produced
<i>Vespa vulgaris</i>	Normal	September	5,300	11,000
	Giant	October	11,600	20,000
<i>Vespa maculata</i>	Normal	September	1,150	2,100
	Large	October	3,200	5,000
<i>Vespa crabro</i>	Normal	September	1,700	3,600
<i>Polistes gallicus</i>	Normal	September	60 (120)	120 (240)

These estimates are based on actual observations (Janet, 1895, 1903; Ritchie, 1915), and are probably accurate for healthy colonies under normal conditions. They may be surpassed under favourable conditions.

(5) Conclusions

The outstanding fact in the life of wasp colonies (in the Holarctic region at least) is their low longevity. They all develop and die in one season, mainly lasting from May to October. The growth is slow during the first $1\frac{1}{2}$ –2 months, when the queen starts building the nest, ovipositing and nursing the first brood. As soon as the first workers appear, they take over the building of the nest and most, at least, of the nursing of the brood. During the following two months the colony reaches its peak rapidly. The queen dies and her death induces the subsequent disorganization of the colony, which begins and, as a rule, is ended *before* the bad season would bring about this disorganization. The males and the young females appear from mid or late summer on. The latter become fertilized, disperse and hibernate in various refuges. The males as well as the workers die before winter. The most sensitive stage of the colony is hibernation. Those females which survive until the next spring generally succeed in founding a new colony. The size which the colony may reach during the season depends mainly on its success in the early period of colony foundation. Environmental conditions, especially temperature and rains, influence it a great deal.

IV. HUMBLE-BEES

The humble-bees (Bombidae) are near relatives of the bees which form small colonies only. As in most social wasps the colony dies out at the end of the autumn and the fertilized females hibernate. These queens begin to build their nest in the soil, earlier in the spring than do the wasps. The queen begins to build several small central cells (when not using an old deserted nest from the previous season), into each of which she deposits 7–30 eggs. The cells are stocked with honey and pollen for the early larval development and the older larvae are fed later on by the workers. The first helper females are very small, as large numbers of them are forced to develop in one single cell. Later on, only 3–7 eggs are as a rule laid in each cell. The helper females are smaller than the true females, but not distinguished from them as the bee workers are from the queen. The absence of males at this period condemns them to sterility. The helper females which hatch later are larger. Unfertilized eggs alone, from which drones hatch, are laid during a certain period in the summer. The eggs laid later give rise to the true females.

Drone eggs may occasionally be laid by unfertilized helper females. The queen dies during the summer or autumn and the colony perishes in the same way as does that of the wasps. The "normal" sex ratio in an autumn nest is approximately 15 per cent females, 25 per cent males and 60 per cent workers.

Studies on the first seasonal appearance of the sexual forms of humble-bees in Germany have been made by Friese and Wagner (1909). The males usually appear two months later than the first females.

The size of humble-bee colonies is generally from 50 to 300 individuals. Réaumur (1740) stated that the colonies are never as populous as they would be expected to be from the number of cells. He observed one nest of 150 cells, in which never more than 50–60 adults lived contemporaneously. The marked loss of helpers by death is always balanced by new additions. No reliable data are yet known on the duration of development, longevity, fertility, total egg production, etc. We may assume, however, that the seasonal population trend must be very similar to that in small wasp colonies.

The number of inhabitants of European humble-bee nests has been studied by Hoffer (1882–3) and Friese (1923). They vary from a few up to 600 cells and from 50 up to 900 adults contemporaneously per nest.

The sketch of the life history, given here, is only correct for most European countries. No colonies of certain Arctic humble-bees, as *Bombus kirbyellus*, have ever been known. In other species the colonies in Arctic regions are extremely small because the vegetation period is restricted to 4–8 weeks. In more moderate climates the colonies are not destroyed during the winter, even if brood rearing is interrupted at that season. Thus, humble-bee colonies in Corsica do not perish in the winter, but during the prolonged drought of summer (Stellwaag, 1915). In the subtropical climate of São Paulo, Brazil, the colonies of many humble-bees do not perish in the winter, but interrupt the brood-rearing cycle. Even in the winter, these bees fly about on warm days, whereas other species perish there every winter. The colonies of the perennial species may grow to considerable size. Ihering (1903) counted 475 individuals in one nest, 876 (450 females, 271 males, 155 workers) in another.

The lack of relation between the number of cells and the number of individuals in the nest is even greater than in the wasps. Many cells do not even serve as brood cells but as honey stores, and a varying number of eggs may be bred in one brood cell, even contemporaneously (Taschenberg, 1878).

V. SOCIAL BEES

(1) *General biology of bee colonies*

It is interesting to follow the development of colonies through the many presocial stages of the solitary bees. This is not the place to dwell upon these fascinating developments (which have been described by Friese (1923), Wheeler (1926 *a* and *b*) and others) at length. All really social bees (Apidae) form populous colonies, which dwell in open nests in the tropics and in hollows of old trees or

even in clefts of rocks in other regions. The nests persist for many years and reproduction takes place by partition. At the population peak a large percentage of the colony may swarm out together with, or followed by, the young or old queen. The swarming bees aggregate in a cluster and enter a suitable new breeding locality. Copulation occurs only once in the life of the queen, during a special nuptial flight. The workers are genotypically females which by special feeding have been bred to workers, whereas the queen larvae are fed with different food. The males originate from unfertilized eggs. Honey and pollen are stored for the unfavourable season, at which time—in temperate regions—the brood-rearing cycle is interrupted. This is not the case under warmer conditions. Even in the coastal plain of Palestine the breeding activity is, as a rule, not interrupted (Buttel-Reepen, 1903-1915; Zander, 1913; Snodgrass, 1925; Bodenheimer & Nerya, 1937).

(2) *Quantitative data on bee colonies*

Probably the oldest population census of the honey-bee was carried out by Swammerdam (1737). In his famous book *Biblia naturae* he records counts of 3 Dutch straw-hives.

The first hive was 1 year old and opened on 10 March. It contained 22,579 cells in 9 combs. Bees had been reared from 7824 cells during the last season, this number representing the year's minimum production of bees. The remainder of the cells contained honey and pollen.

On 14 June a swarm left a hive. The swarm contained 1 female, 4 drones and 2433 workers. The population which remained in the hive comprised 1 female, 693 drones and 849 workers; 14 sealed queen cells, 858 cells with sealed drones' brood and 6468 sealed workers' brood. The adult population was 11,626, 7340 advanced development stages being present contemporaneously.

The third comprised a swarm which occupied the hive on 25 June and was studied 6 days later. It contained 1 female, 33 drones and 5635 workers. The brood counted 45 eggs, 150 larvae and 3392 ordinary brood cells with honey.

We only possess scanty knowledge of the populations of the various kinds of primitive beehives and it is most important that data concerning them should be collected before these primitive beehives have disappeared. It seems that all indications in modern bee literature refer to beehives of the modern type (Langstroth, etc.). During hibernation the colony may be reduced to 10,000 worker-bees or less. Up to 15,000 workers may survive under good conditions. Normal beehives have 30,000-40,000, very good ones occasionally 70,000 adults contemporaneously at the population peak. The total quantity of brood, even during the height of the season, only reaches 40,000 in very exceptional cases. Most of these individuals are workers. The quantity of drones is very different, assumingly based on racial differences, and ranges from 50 to 5000 per colony. The natural losses of the colony by swarming are treated later.

The bees use the same cell for breeding purposes repeatedly during the season.

We possess very few reliable data on populations of tropical bees, on the enormous clusters of *Apis dorsata* and on the colonies of the tiny *A. florea* from the

Oriental region. Friese (1902) counted 7000 worker cells, 300 drones and 5 queen cells in a nest of *A. florea* (in addition to 1400 honey cells). The same author estimates a brood comb of 1 sq. m. surface (the normal size) to contain 70,000 cells. The colonies of the South American stingless bees are populous. In *Trigona* 50,000–100,000 adults may be present in one nest. The largest nest of *Melipona* studied (belonging to *M. postica*) contained 27 combs with about 64,000 cells and 70,000–80,000 adult bees. Normally, though, the nests of *Melipona* count 500–4000 adults.

(3) *Dynamic analysis of the seasonal population trend in a colony of the honey-bee*

(a) *Ebert's approach to the problem.* The only previous attempt to study the dynamics of bee populations known to the author is that of Ebert (1922). He divides the bee population into three categories: brood, young bees and field-bees. The number of bees of each category is estimated by the planimeter and by weighing. For exact method the reader is referred to the original.

These observations are made at intervals of 21 days. A second series of observations with 21 days' (= duration of brood development) interval may be intercalated.

This system is fairly exact for the total quantity of brood throughout the season. It yields both the total seasonal egg production of the queen and, by

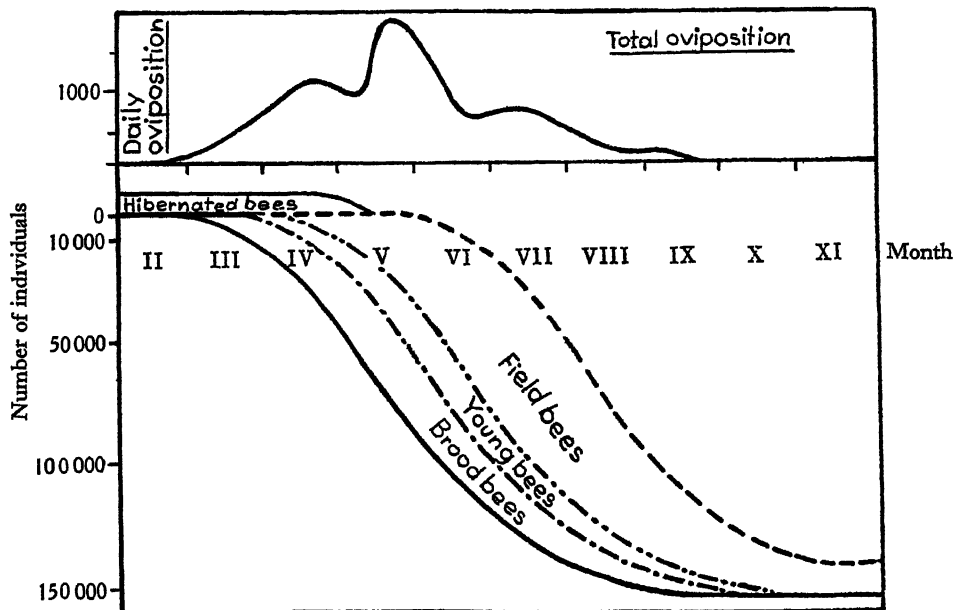


Fig. 6. Scheme of the seasonal population trend of a bee colony (after Ebert).

additional calculation, the daily oviposition. An idealized scheme of the annual development is reproduced in Fig. 6 for a colony with 10,000 hibernated bees, 152,000 total egg production from February to September and an average longevity of field-bees of 7 weeks.

Many practical applications of this type of research are emphasized by Ebert, but his suggestions do not seem to have been followed.

Further research in the biology of bees, and especially the work of Nolan (1925), Roesch (1925, 1927) and Morland (1930), enable us now to use a simpler system in evaluating the population trend of a bee colony, probably with greater accuracy.

(b) *Method of calculating the seasonal dynamics of a honey-bee colony.* Premises: (1) The calculation is based on the consecutive division of labour (Roesch, Morland), which on the average results in the following scheme:

	Workers (days)	Males
Development: Egg stage	3	3 days
Larval stage	6	6 days
Sealed brood	12	16 days
Adult life: Nurse-bee	10	100 days (3-4 months) (in winter longer)
House-bee	10	—
Field-bee	22	—

The stages of adult life are to a certain degree modifiable and the house-bees especially may serve somewhat longer as nurses, or be speeded up to serve as field-bees. They are therefore called "control bees" by some American authors.

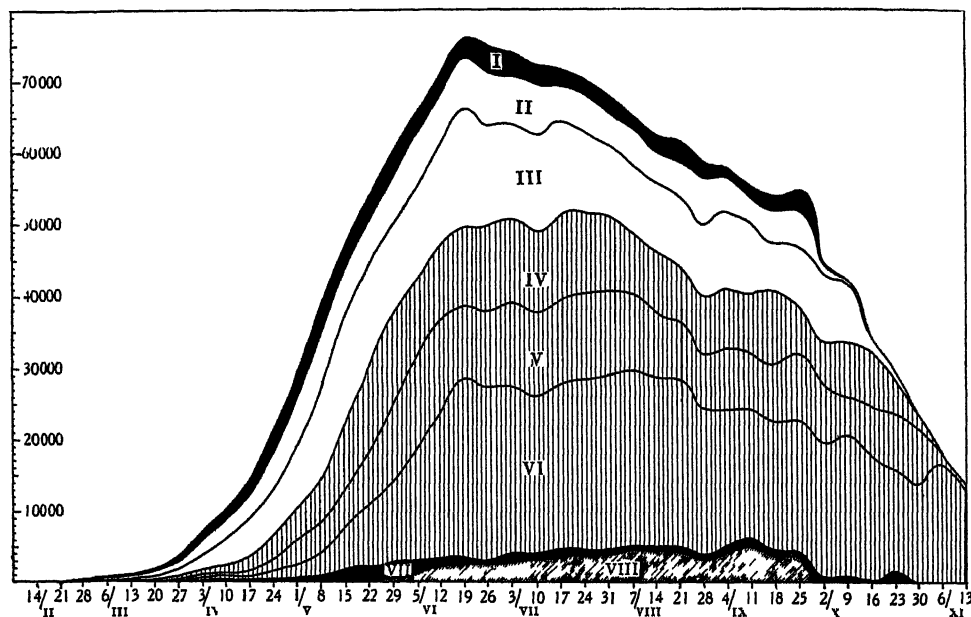


Fig. 7. The population trend of a colony of Cyprian bees (computed from the observations of Nolan, 1928). I-VI, worker; VII-VIII, drones: I, eggs; II, larvae; III, nymphs; IV, nurse-bees; V, house-bees; VI, field-bees; VII, drone brood; VIII, adult drones. The ordinate indicates the number of individuals in every stage, the abscissa the date.

(2) Any successfully developing egg does not, under normal conditions, show later mortality. (3) The fertility of the queen does not differ from one day to another,

but only changes gradually. The procedure will be published elsewhere *in extenso* (Bodenheimer, 1937). It is based on observations of the total brood, at intervals of 21 days, or the sealed brood, at intervals of 12 days.

(4) *Swarming, fertility and longevity of bees*

(a) *The foundation of the colony (swarming)*. The foundation of a new colony in the honey-bee is never done by single fertilized females but by a division of the existing colony, which is accompanied or preceded by the breeding of new queens. The method of colony foundation nevertheless shows great variation (Goetze, 1930 a). The *Melipona* bees of South America send the young queen with the departing swarm. The Indian giant bee (*Apis dorsata*) sends a swarm of workers which builds the new nest and the (young?) queen follows later. In the European honey-bee (*A. mellifica*), and probably also in the tiny Indian bee (*A. florea*), it is the old queen who leaves the colony with the main swarm. Additional after-swarms are accompanied by supernumerary young queens. A very peculiar type of behaviour is reported by reliable observers for the Cape bee. These often swarm without a queen and the workers are able to breed their own queen parthenogenetically. A more thorough study of this bee is urgently needed.

The size of the departing swarms is considerable. Apart from other factors, it depends mainly on the size of the parent colony. Swammerdam (1737-8) reports on a swarm of about 5700 bees. Butler (1623) estimates a very large swarm to contain 26,880 bees, a large swarm 22,400 and a mediocre one 17,920 bees. Réaumur (1740) counted 43,008 bees in a large swarm. In good colonies in modern hives 27,000-36,000 bees are supposed to leave the parent colony in the main swarm, and 10,000-15,000 in the first after-swarm, if there is one; a greatly decreased number succeeding after-swarms.

The new swarm settles speedily in its new home and begins to build the nest. The report of Swammerdam gives a good idea as to the intensity with which the building and brood-rearing activity in the new nest proceeds.

Goetze (1930 a) is quite correct in stating that the abundance or lack of suitable natural nesting localities is the primary limiting factor in the number of bee colonies, in moderate climates at least, and that not 1 per cent of the existing colonies could survive in these regions if left to themselves. But the quantity of food present during the unfavourable season is certainly quite as important (Bodenheimer, 1928). Another limiting factor is the climate at the time of the nuptial flight. During warm dry weather in 1929, 95 per cent of queens near Landsberg mated successfully, whereas in the humid year 1927 only 60 per cent did so (Goetze, 1930 b). Hibernation is always a critical period. The Egyptian bee dies in the winter of a temperate climate, because it does not form a sufficiently dense cluster.

Swarming occurs always at the seasonal peak of the development of a colony. It must be provoked by objective conditions and it spreads rapidly through the colony. Adults of all ages above 3 days participate in the flight, but the house-bees seem to be somewhat preponderant.

The causes of swarming have been widely discussed, but it has been generally

agreed that relative overpopulation of the nurse-bees is at least a factor which regularly precedes swarming. "Overcrowding of the hive, lack of ventilation, heat, abundance of drones and similar conditions which often prevail at the time that swarming is instituted have been given as causes or contributing conditions to swarming, but unfortunately for these speculations, these conditions may be wholly or partially lacking at the time of swarming and cannot, therefore, be considered as actual causes" (Phillips, 1928).

"Although the cause of swarming has not been definitely determined, the one factor which is universally present in normal swarming is that of a congestion of bees within the brood nest (not elsewhere in the hive). Other factors often mentioned as causing swarming are not universally present. Such a congestion of bees within the brood nest is usually brought about by a preponderance of recently emerged and emerging young bees, but the effect of such crowding may be greatly intensified by numerous factors in the environment, not universally present, which may be considered as contributing factors, but not causal" (Demuth, 1921). Heavy honey flow is certainly not the cause of swarming. But it may stimulate it and its absence may prevent swarming by preventing the colony from growing strong enough to swarm.

A most interesting theory (the "brood food" theory of swarming) has been promoted by Gerstung (1926), who was the first to compare the bee colony to a living organism. The queen is its generative organ and swarming is the act of reproduction. The swarming is induced by a kind of endocrine secretion. The nurse-bees have highly developed lateral pharyngeal glands of the head ("food brood" glands) in which they produce the food for the larvae. When this secretion is much larger than the need of larval food, they start to build queen cells, or, as Morland (1930) puts it, "the surplus is given to certain favoured larvae in order to get rid of it. These larvae develop into queens, and when the cells are sealed, the colony is liable to give off a swarm."

Morland (1930, 1935) continues the reasoning of Gerstung in stating and proving experimentally that this swarming impulse is very strong when the surplus of nurse-bees grows very suddenly, either by artificial addition of many nurse-bees or by a sudden fall in the egg-laying activity of the queen.

A further proof of the presence of a special physiological condition in the colony is the common presence of workers with mature ovaries, which are probably conditioned by changes in the protein food (Tuenin, 1926; Perepelova, 1929). The new method of computation, which has been developed in the previous section, permits of a comparison of the actual relation between brood and nurses in the colony throughout the season (Fig. 8).

There seem to be three recognizable critical periods: (1) When the brood-nurse ratio falls to about 2.5 the first drone eggs appear. (2) When it falls below 1 the colony must be ready to swarm. (3) Some weeks later the number of drones surpasses the minimal density needed for a successful nuptial flight.

The fact that throughout the longest part of the brood period the brood-nurse ratio is actually about or below 1, i.e. that one nurse is occupied with one egg or

larva only, is rather surprising. But Morland is correct in assuming that it is not this ratio itself which induces swarming, but only its rapid decline. A low brood-nurse ratio in itself does not induce swarming of the colony. The bee keeper's task must therefore be to counteract, by manipulations, any rapid falling off of egg laying by the queen.

In relation to these facts the survey of Lineburg (1924) on the nursing activity of the honey-bee is of interest. He reports a total of 10,412 visits of nurse-bees to

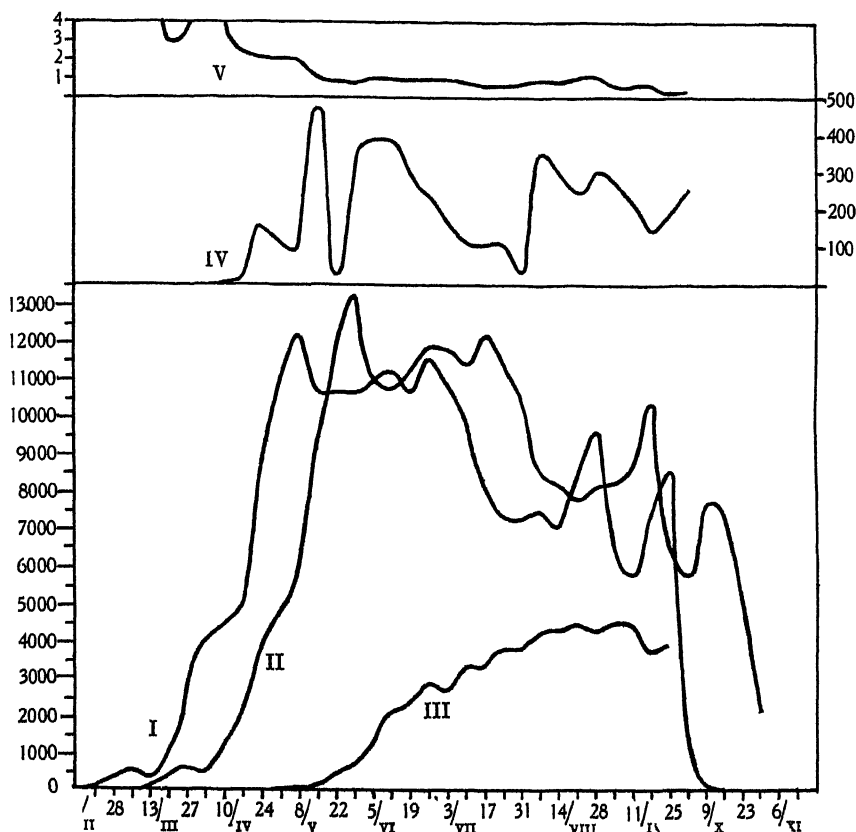


Fig 8. Analysis of some seasonal relations among the different ages of a colony of Cyprian bees (Nolan, 1928). I, eggs + larvae present (brood to be nursed); II, nurse-bees present; III, adult drones present; IV, drones' eggs; V, relation between $\frac{\text{eggs + larvae}}{\text{nurse-bees}}$. The ordinate indicates the number of individuals in every stage, the abscissa the date.

eggs and larvae during the egg and larval instar, with a daily average of 1300 visits. During the 8 days of nursing 12.7 per cent of the time, or $24\frac{1}{2}$ hours, are devoted to actual visits.

In view of these facts it seems unjustified to accuse the bees of laziness, as has recently been done (Frisch, 1927). The larval nursing is interrupted by profuse secretion of the brood glands of the nurse, which undoubtedly has a marked effect on its

metabolism. The house-bees must likewise produce wax abundantly and the field-bees' number of trips is limited.

Swarming is preceded by rearing of queen cells when an old queen must be superseded. In this case, as well, the relative lack of brood (i.e. the population pressure of nurse-bees) presumably induces the rearing of new queens.

(b) *Fertility.* It is Swammerdam (1737) again, the discoverer of the female nature of the queen bee, to whom we owe the first data on the fertility of the queen. He states that 17 eggs are in every one of the 150 ovarioles of each ovary, which allows for 5100 eggs per female. The first data on the oviposition rate of the queen were given by Réaumur (1740), who reported that at the peak of the breeding

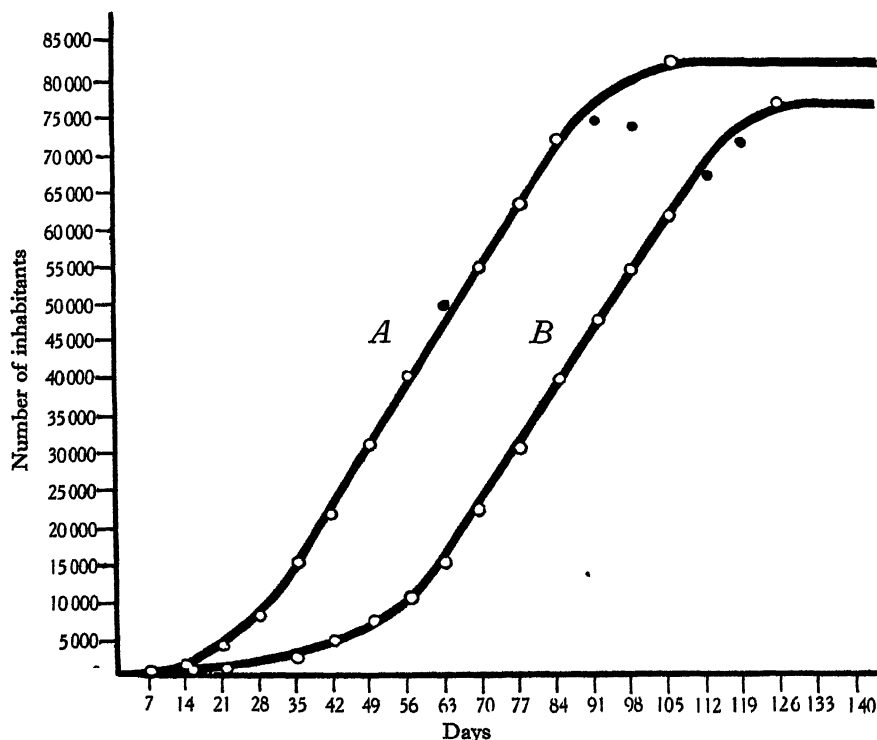


Fig. 9. The growth curve (logistic curve) of a Cyprian bee colony (B, 1928) and that of an Italian bee (A, 1925) in the same apiary.

season, during two spring months, an average of 200 eggs may be laid by the queen daily. These figures were accepted as correct for almost a century.

In the second half of the last century Berlepsch's (1860) findings dominated. He counted 3021 eggs laid during one day by one queen and she had oviposited during the preceding 20 days at a rate of 3000 eggs daily. But Berlepsch himself realized that this figure was unusually high and that an average of only 1200 eggs would be nearer the usual number. The queen mentioned was active for 5 seasons and must have laid at least 1,300,000 eggs during her life (cf. Baldrige, 1861).

The first complete records over a whole egg-laying season were published by

Desborough (1852, 1855, 1868) whose counts were based on a colony below normal strength. Baldensperger (1895) was the first to report on the total seasonal egg production by colonies of normal strength. Dufour (1901) and Bruennich (1922) made important surveys, the first by actual counts, the latter by calculating brood areas. It is now generally agreed that this latter method, which is speedy and easy to use, gives results reliable enough for most purposes.

The total quantity of eggs laid by a queen under favourable conditions in the modern beehive varies somewhat with locality, age of queen, climatic conditions of the year, etc. In 25 colonies from various seasons, on which Bruennich reports, the average total egg production was 116,333, ranging from 65,770 to 175,000. Nolan's (1925) colonies were stronger, but the average difference is probably much smaller than that of the two best colonies, which produced 190,750 and 186,969 individuals. Ebert (1922) regards an annual production of 150,000 bees as desirable.

But this actual total production is not really the total egg production of the queen. "It has long been recognized that a queen bee lays more eggs than ever develop into adults. This excess of eggs is often very evident in spring, becoming less apparent during maximum brood-rearing activity, and again becoming evident in the fall" (Nolan, 1925). It is highly desirable that this phenomenon should be studied more closely, in order to ascertain its numerical extent and its causes. It is fortunate, however, that apart from this mortality in the early egg stage all instars develop without any important mortality (under normal conditions).

With regard to the rate of oviposition, it is known that the daily egg-laying rate is small at the beginning of the season, generally rises quickly to its peak and falls slowly during the rest of the season, 1500–2000 eggs per day being the maxima at the height of the season. However, Baldensperger (1895) reports an average daily oviposition of 2600 eggs during a 23-day period and Phillips (1928) observed 5000 eggs per day per queen under exceptional conditions, which are never realized in a normal colony. The maximum oviposition per day (of 12-day periods), under normal modern conditions, was in the 29 colonies of Bruennich (1922) 1395 (900–2000) on the average during various years. The average per day (of 21-day periods) for 21 colonies of Nolan (1925) was 1203 (905–1587). In colony 4.1921, where the smoothed average maximum was 1587 eggs per day, the real maximum daily oviposition was 2148 (on 8 June). In good to normal colonies a daily oviposition rate of 1000–2000 eggs can be expected during the period of rapid expansion (i.e. eggs which successfully develop). All other indications must be regarded as very exceptional or exaggerated.

With regard to oviposition speed we have only one very incomplete observation. Phillips (1928) followed the oviposition of one queen: 4–6 eggs were laid per minute, 20–25 min. of egg laying being followed by a 5 min. rest. This would yield 6000 eggs per day, which indicates that long periods of rest are intercalated or that the oviposition rate is not permanently maintained at that level.

The total egg production of a normal queen is estimated by Cheshire (1885) to be 1,500,000 and 4,000,000 sperms are stored during fertilization in the receptaculum seminis. This number is certainly not the normal average. In assuming an average

longevity of even 4 years for the queen and taking into consideration a total annual production of 100,000–200,000 eggs, the normal fertility of a queen would be 400,000–800,000. This figure could probably be exceeded under exceptional conditions (long life duration, high fertility, very favourable environmental conditions) until it reached Cheshire's estimate. However, no definite solution can be arrived at until the quantity of non-developing eggs has been studied.

(c) *Longevity*. The longevity of the honey-bee is a very complicated physiological problem, which has not yet been adequately treated. The longest living caste is the queen. Normally, she survives 3–4 seasons. In the second year, as a rule, she reaches her highest fertility, which is often maintained during the third and perhaps occasionally even the fourth year. In many cases the fertility is lowered from the third year onward. A few exceptional cases of 7 years' longevity and one of even 9 years have been reported (Phillips, 1928).

A confirmation of Nolan's (1925) conclusion that a strong colony tends to remain strong is supplied by Bruennich's (1922) data.

The drones have a physiological longevity of 3–4 months, but they die soon after mating and are often driven out prematurely from the hive by the workers at the close of the honey flow. Left to themselves they soon perish.

"The worker bees, which develop from eggs identical with those from which queens issue, live 6–10 weeks in summer and possibly 6 months or longer in winter. Those which emerge in time to take part in the gathering of a heavy honey crop usually live about 6 weeks, but if no nectar is available the length of life is extended. Those workers which emerge at the close of summer are the ones which must live until the following spring if the colony is to survive, for there is no rearing of brood in normal colonies in winter. It is obvious that the length of life is influenced to a marked degree by the amount of work which they are called upon to do. Similarly queens live longer if they are not compelled to lay such large numbers of eggs. If bees winter badly, so that they are compelled to produce much heat, they often die, in the spring, faster than they are replaced by oncoming bees, a condition known as spring dwindling. Not all bees die in summer within 6 weeks of their emergence, for if all brood rearing is prevented, it may happen that the last bee will not die for 4 months. All these facts indicate that a bee is born with a definite amount of ability to do work and when its energy is expended the bee dies. It must not be concluded that bees have no recuperative power, but it is obvious that their term of life is limited by the amount of work they do" (Phillips, 1928).

The actual longevity of worker-bees at the height of the season is lower than is often maintained. Morland (1935) states that after about 21 days 50 per cent, after 40–50 days 100 per cent, of all bees marked immediately after hatching had disappeared (cf. also Eckert, 1933).

A limited number of trips per field-bee (about 30; Lundie, 1925; Bodenheimer & Nerya, 1937) agrees well with Phillip's (1928) conception of the limited power of total activity.

The longevity of the honey-bee depends greatly on its social behaviour. The field-bee which is on the flight is exposed to sun radiation and has therefore a body

temperature reaching or surpassing 35°C . In the shade, however, at night, it would have a temperature about equal to that of its environment. In the hive the body temperature of 35°C . is retained. The longevity of solitarily kept bees is always inferior to that of even small groups of 15–20 bees. The group forms a cluster during inactivity and thus economizes a considerable loss of heat by radiation. Its metabolism is lower than that of the single individuals, as Kalabuchov (1933, 1934) has pointed out.

The longevity of winter bees may rise by suppression of the normal development of the brood-food glands and wax glands. A study of this question is required. Possibly only those workers hibernate which do not find any more brood. Fig. 7 suggests this hypothesis and supports it quantitatively.

(5) *Conclusions*

Bee colonies are always populous and reproduce by swarming. Provided that suitable breeding niches are present, reproduction shows little mortality. The number of fertile females to be bred in order to maintain the species is correspondingly small. The fertile female is produced whenever an old queen is to be superseded, owing either to death or to having ceased to be sufficiently fertile. The number of males is small and they do not participate in the work of the colony, except by their sexual function. When this is accomplished, they are driven out of the hive and die.

The period of seasonal activity is interrupted by a period of inactivity during the winter in temperate climates, when no brood is reared and no food is present. The active period starts with a progressive increase which follows a logistic curve. The oviposition rate is then reduced and the population level is maintained at a more or less high level with several declines and minor peaks which depend on environmental conditions (weather, nectar and pollen crop, etc.). At the end of the active season the breeding stops rapidly. Nolan (1925) calls these three main periods the period of initial expansion, the major period and the period of final contraction. Weather, nectar and pollen crop, etc., influence the breeding activity in a direct and indirect way. Under the latter head it may be mentioned that during a very good honey crop most cells may be filled and that the queen does not find a sufficient number of empty brood cells. Unfavourable winter conditions leave the colony weak at the beginning of the new season, so that the initial expansion is subnormal. If there is too much brood rearing during the major period, we find a useless consumption of stores, etc.

By the method described above it will now be easily possible to follow the seasonal population trend of bee colonies and it is hoped that this may lead to a series of quantitative observations under controlled or known environmental conditions. The writer has started such work in Palestine. In this way it will be possible to obtain a clearer analysis of the quantitative population trend in bee colonies and their causes. Comparisons between bees in different climatic regions will extend the results.

VI. GENERAL CONCLUSIONS

(1) *Reproduction*

There are many ways of reproduction of social insects and their colonies. Primarily, the reproduction in all social insects was sexual. Regarding the colony as a single organism, the sexual forms correspond to the eggs and sperm. These true sexual forms only appear after the colony has reached a certain age. The sexual forms are always winged. They leave the nest for the nuptial flight which, as a rule, separates them definitely from the parent colony. The sex ratio varies. Either the copulating pair or the female partner alone is in a condition to found a new colony. Their initial mortality may be compared with that of the seeds of a tree. In wasps and humble-bees the colonies die every year and the new fertilized queens hibernate as do the winter eggs of certain plankton animals or of aphids in a temperate climate. In the most populous colonies of ants, bees and termites we find another facultative method of reproduction, which may be called asexual. Parts of the population segregate and found a new colony in the neighbourhood, which may reproduce its own supplementary sexuals (termites), adopt young sexuals, often of the same colony (ants), or take them with it (bees, some ants). The paucity of sexually reproducing forms, as well as the faculty of producing sexuals (by differential feeding, or the development of unfertilized eggs) when the maintenance of a still vigorous colony demands it, should also be mentioned. The colonies of wasps, bees and ants are primarily monogynous.

(2) *The logistic curve*

Pearl (1926) has shown in his comprehensive studies that the logistic curve represents the growth of organisms as well as of populations. The growth of all colonies analysed in this article follows a logistic curve very closely. It starts with a slowly increasing initial period, passes through a second period of rapid growth and maintenance (vigour) and is followed by a period of contraction or senescence.

The agreement with a logistic curve is best seen when $\log \frac{k-y}{y}$ is plotted against x of the equation $y = \frac{k}{1 + e^{a-bx}}$.

The closer the points approach a straight line, the better they fit to the logistic curve.

It is evident from Fig. 10 that the first and last parts of the growth of colonies of social insects often show deviations, whereas in about 80 per cent of the extent the points fit very well. In the initial period the colony is still enormously influenced by environmental factors, whereas with the approach to the stable maximum the destructive tendencies begin to show their first signs of influence.

The logistic curve covers the seasonal growth of bee, wasp and humble-bee colonies, as well as the perennial growth of ant and termite colonies.

Besides environmental factors there are internal (genetic) factors which influence the population growth, such as differences in size and longevity of colonies of the

same species. Large colonies grow quicker from the very beginning than do the small or average colonies.

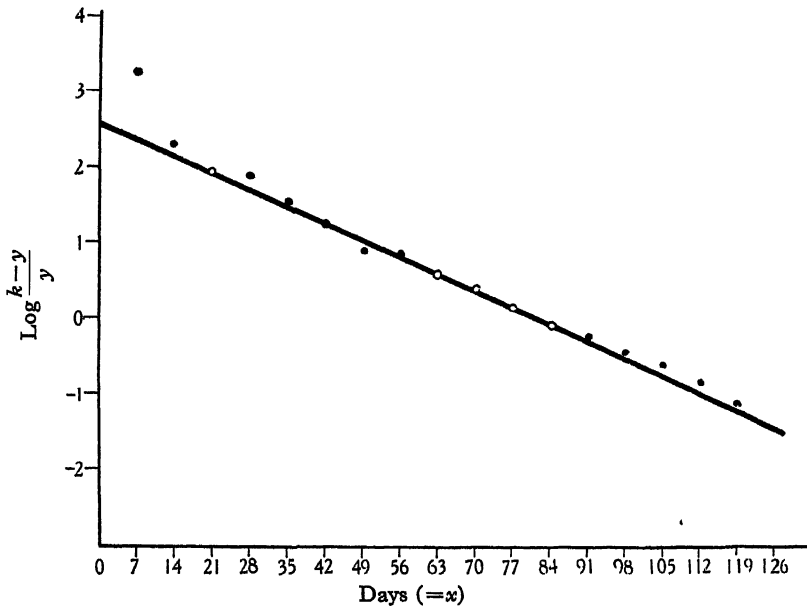


Fig. 10. The logarithmic fitting with the straight line (Cyprian bee colony, 1926).

(3) *The initial period of colony foundation*

The initial period of colony foundation starts with the nuptial flight and ends with the rearing of sufficient workers or helpers to free the queen from all "trivial" work in the colony and to devote herself mainly to oviposition. During this period the queen, or the royal pair, has generally to pass, except in tropical conditions, a more or less protracted period of several months to over a year, during which she has to perform every kind of work. She begins to build the nest, nurses the first brood and feeds it, etc. In many cases the queen, after the histolysis of her wing musculature, devours a large fraction of the eggs or larvae, and feeds the other larvae with them. This is very common in ants, where the queen seems to depend entirely on the workers for food and suffers from lack of food until the first of these appear. We also encounter this habit in termites. This destruction is rational as it helps to maintain the colony during a very sensitive stage of its development.

The initial period is doubtless by far the most sensitive stage of the development of the colony. In ants and termites the number of swarming sexuals is often considerable. But numerous enemies attack them from the royal chamber. Numerous sexuals perish because they do not succeed in finding a suitable locality for such a chamber. And among those individuals or pairs which do so, relatively only very few succeed in escaping the perils of the initial period. Enemies, unfavourable micro-climatic conditions and starvation are the main dangers.

In wasps and humble-bees the heaviest toll is levied on the scattered hibernating

queens. In bees, it is the number of successfully hibernated adults (depending on the quantity of stores) which mainly decides the fate of the colony in the following season.

(4) *The period of expansion and vigour*

The outstanding fact, from the point of view of animal populations, is the extremely low mortality which follows the great initial losses. While the workers of the initial period were nanic, they now reach their normal size. Optimal nutrition is secured by the activity of a sufficient number of nurses. And, in addition, many social insects attain a more or less complete homoiothermy for their brood in various ways (Bodenheimer, 1928; Himmer, 1931*). The temperature fluctuations in the brood of a bee nest scarcely surpass 1°C . Ants utilize the sources of heat during the breeding period to the fullest extent possible, as is the case with many wasps and probably with termites. Steiner (1930*a*) has recently given a good survey of the different methods of creating optimal conditions of temperature for the brood. While in bees the brood lives practically in a good thermostat, in wasps and ants (in temperate climates) the optimal temperature is maintained only during the sunny hours of the day (Bodenheimer, 1934). There is little doubt that what is true for temperature holds also for air humidity. The difficulties of measuring it have up to the present prevented the exact study of this factor.

The extremely low mortality of the brood, due to environmental conditions approaching the optimum, is the main advantage of social insects over solitary species. This advantage makes calculations possible, provided diseases or extremely unfavourable environmental conditions are absent. In temperate climates hibernation is the most sensitive period, but for humble-bees in Corsica it is the hot, dry summer. Winter is always passed in a state of lowered activity, especially by interrupting the brood-rearing cycle. In bees, hibernation occurs in a state of semi-torpor with the maintenance of a high temperature. In ants we may find simple hibernation, which is ended or interrupted by fine sunny days (as is also the case in wasps and humble-bees), or a true diapause, from which no environmental stimuli awake the animals before a certain time has elapsed (Holmquist, 1928*b*). The first appearance of sexual forms, at least in normal numbers, rarely occurs before the turning point of the logistic curve is reached. It is a sign of approaching or attained maturity.

(5) *Period of senescence and death*

When the growth of the colony has reached its maximum, the maximal population level may be maintained or it may slowly fall. The maximum does not ever seem to be maintained for a very long period. In nearly all well-studied colonies the maximum is followed by a slow contraction, which leads, after a generally very limited period, to the death of the colony. This death is conditioned in all strictly monogynous colonies by the death of the queen, as has been clearly demonstrated for wasps. This is true also for humble-bees, many ants and bees. The longevity of the queen determines in this case the longevity of the colony.

* *Biological Reviews.*

In termites, on the other hand, with their neotenic supplementary sexuals and in the adoption colonies of ants and termites, there might be an unlimited existence. This potential immortality is seemingly never utilized, and from *Formica* mounds as well as from the *Termes* hills we know that depopulated and deserted colonies are quite common. The age of colonies which have been carefully observed rarely surpass 15 years and the maximum of 80 years remains within the limits of human longevity. This limited duration of life of the colonies of social insects is probably the strongest reason for regarding them as organisms. The potential immortality is never realized.

(6) *The growth of organisms and of animal populations*

The growth of organisms and of animal populations under experimental conditions, as well as that of human populations, has been known to follow a logistic curve since the classical studies and experimental work of Raymond Pearl.

The fact that the growth of plants or poikilothermal animals living under standardized environmental conditions, and of homoiothermal animals, follows a standard regularity, did not arouse any special attention, because planned reaction is generally regarded as one of the outstanding phenomena in the organization of living organisms.

As soon as plants or poikilothermal animals are subjected to changing environments their growth curve becomes irregular and deviates from the logistic curve. When the changes are cyclic and regular, the growth may still follow a logistic curve, providing the time units are chosen in a suitable manner. But when the environmental changes are irregular, the growth also becomes irregular. These same rules hold for animal populations.

Every growing animal population studied so far under experimental conditions (from Protozoa to mice) has shown a high correlation with the logistic curve. The scarcity of growing populations which follow the logistic growth under natural conditions is due to the scarcity of environments which are constant for a sufficiently long period.

However, even in social animals the logistic curve is not valid throughout the whole run of the population growth. In Fig. 10 we find a very good agreement between the curve and the major period of seasonal growth of a bee colony. But deviations are recognizable at the beginning, and to a smaller degree at the end, of the period of growth. This is easily understood, because at the very beginning of the seasonal growth the queen, as well as the whole colony, still depends to a very large extent on the environmental fluctuations, especially climate, nectar and pollen crop. During the whole growth period, such external factors may influence the progress of the increase of the colony. This is illustrated by Fig. 9, showing the growth of another bee colony, hampered to a small degree by unfavourable environments. However, owing to the high constancy of temperature, humidity and larval nutrition, existing in the bee colony after the sensitive initial period has been passed, the seasonal development of the bee colony approaches that of an homoiothermal organism. Fig. 5, illustrating the growth of a colony of *Vespa crabro*, which depends

to a larger extent upon environmental influences, shows a much stronger deviation from the logistic curve in its initial phase, and considerable disagreement even during the major period. During this period the growth was repeatedly disturbed by unfavourable climatic conditions. In the subtropical colonies of *Kaloterms minor* we find the same deviations, whereas they are absent in the tropical colonies of *K. tectoniae*, which live under more constant conditions. All these initial and final deviations point in the same direction: in the beginning the growth is quicker than calculated, at the end, slower. Similar deviations, especially the initial ones, can be observed when animal populations are experimentally grown in free spaces.

The general occurrence of these deviations may easily be explained. At the beginning of population growth, the primary tendency is towards a simple exponential increase, corresponding to the theoretical ideal curve of increase. But at a very early stage of the population growth the limiting influence of organized or organism-like growth makes itself felt and forces the simple exponential increase into a logistic one. In populations growing in a free space it is the limitation of the space which makes itself felt and leads to the same consequence. The final deviation, which is not so general and clear-cut a phenomenon, is the expression of the high environmental resistance produced by the approach to the maximal population density. The strong resemblance between an organization of colonies of social insects and the life cycle of a living organism itself is therefore an important premise for their logistic growth.

(7) *Various results*

(a) *Longevity.* One of the fundamental problems requiring further observation is the longevity of all stages. While the longevity of the sexuals is more or less known, we lack exact evidence on that of the workers and soldiers. We only know that ants and termites can live for one or more years, whereas the life of bees, humble-bees and wasps is normally restricted to some weeks. But it seems that, at least in bees, there exists a reverse correlation between life duration and activity. It may be that the longevity of the hibernating bees (up to 6 months) is induced by the lack of nursing and wax-secretory activity, which certainly has a greater claim on the metabolism of the bee than has hitherto been realized. This theory is in agreement with the observations of Phillips (1922) on the limited amount of working capacity of the normal worker-bee and its relation to longevity.

(b) *The division of labour* among the working castes is based on two different principles. In termites, and often in ants, every occupation has its morphological equivalent. In the bees every adult worker has to fulfil each function successively. She has to work for about 10 days as nurse, for another 10 days as house-bee and the rest of her life as field-bee. The first two stages are conditioned physiologically by the contemporaneous development of the brood, or the wax glands. The recent discovery of well-developed protocerebral glands in young field-bees (Weyer, 1935) makes it probable that also the third fundamental change in occupation is induced by hormonal secretion. In wasps, humble-bees and some ants a very primitive begin-

ning of successions is superposed by contemporaneous work of most kinds by workers of very different sizes.

(c) *Number of cells and adult production.* In wasps, humble-bees and bees, a fixed correlation between the number of cells and the number of inhabitants or even of the total adult production does not exist.

(d) *Territories.* In ants, and probably in termites, definite territories are occupied by every colony. Elton (1932) reports on the division of a *Formica* territory after a daughter colony had been segregated. These territories were mainly protected against members of the same species. The division of the territory, as observed by Elton, occurred without any struggle. In bees, wasps and humble-bees no territories are known. Many nests are often found in close proximity.

VII. SUMMARY

1. The nests of termites contain from a few hundred to several million inhabitants. The daily oviposition of their queens ranges from 1/20 to 43,000 per day. One, or less frequently several, royal pairs which copulate often are the reproducing caste. The growth of colonies in some *Kaloterme*s species is analysed. Potential immortality and the sensitive stages of growing colonies are discussed.

2. The nests of ants contain from a few dozen to about 100,000 inhabitants. In the early stage of colony foundation most eggs are eaten by the queen or fed to the first developing larvae. The colonies are generally monogynous, rarely containing several queens. Other types of colony foundation are discussed. During the nuptial flight the males die soon after the single copulation. The age and sensitive stages of growing colonies are dealt with.

3. The nests of social wasps contain from a few dozen to about 4000 adults. They are monogynous and as a rule live through one season only. The growth of a colony of *Vespa crabro* is discussed. The number of cells is always lower than the total annual production. Copulation takes place in autumn and the fertilized females hibernate.

4. The nests of humble-bees contain from a few dozen to about 1000 adults. Their seasonal history is similar to that of the social wasps.

5. The nests of social bees contain up to 80,000 adults at the peak of the seasonal development. They are monogynous, but may live through several seasons. The seasonal development of a normal colony of the honey-bee in a moderate climate is described. The fertility of the queen, the reasons for swarming and the longevity of the different castes are dealt with.

6. The growth of colonies of all social insects follows a logistic curve and may be divided into three main periods: (1) the initial period of colony foundation, (2) the period of expansion and vigour, (3) the period of senescence and death. The growth of these colonies resembles that of organisms.

The manifold ways of reproduction in social insects are discussed, as well as longevity, division of labour and the foundation of territories.

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ADDENDUM

It was only during proof-reading that I became acquainted with Wheeler's discussion on colony foundation in ants (1933). The independent formicid method of founding colonies is discussed completely and authoritatively.

A few additional interesting types of colony foundations by Formicinae may be mentioned. The Oriental *Oecophylla smaragdina* starts oviposition openly on a leaf. The

nest can only be woven later when larvae are available. In the African *Carebara* ants the swarming giant female carries a few of the minute workers attached to her tarsal hairs to aid her in bringing up her first brood.

Wheeler's observations on the archaic sub-family of Ponerinae, made during two expeditions to Australia, are most important. The bull-dog ants of the genus *Myrmicia* nest in the ground, and each colony contains up to 200, mostly less than 100, adult individuals. They have regular and spectacular nuptial flights. The dealated females live in small chambers beneath a stone and the first workers are nanic. The adults are nectarivorous, the larvae insectivorous as in the social wasps. The nuptial flight of *M. regularis* Crawl. occurs from February to April, but the female may leave its initial nest-chamber and return to it, thus foraging for food. The more abundant food supply in October enables her to lay a number of eggs and to rear a few larvae with insect food, which she captures on similar excursions. Other *Myrmicia* species behave similarly, as does *Amblypone*, which exhibits (probably primary) pleometrosis.

Other species are discussed, amongst them those of *Leptogenys* with ergatomorphic females. In these species too independent colony foundation occurs, which is only made possible by the foraging excursions. It is only in *Brachypona lutea* Mayr that the perfectly claustral type of higher ants is found, which is a specialised condition.

Many interesting remarks on nest foundation in other social Hymenoptera are made. The pleometric type of colony foundation observed in some North American *Polistes* has its origin in the habit of tropical species of this genus of swarming in groups from a destroyed nest and in cooperating in building a new home. The *Halictus* problem is discussed at length. A tabular survey of all types of colony foundation in ants concludes the book.

Interesting observations of Hase (1936) refer to the neotropic wasp *Polybia atra* Sauss. The inhabitants of one nest, counted in September 1930 in Venezuela, were 1528 adults, 233 larvae, 825 pupae and about 900 eggs, giving a total of about 3500 individuals of all stages. Ihering (1904) counted another nest, containing 3882 adult wasps (47 females, 12 of them fertilized, and 12 males).

The bibliographies of the papers referred to above include important additional references.

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THE PASTEUR EFFECT AND ITS MECHANISM

By KENDAL CARTWRIGHT DIXON

(Fellow of King's College, Cambridge)

(Received 15 July 1936)

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I. INTRODUCTION: DEFINITION OF THE PASTEUR EFFECT

CARBOHYDRATES are one of the main sources of energy for living cells. This energy can be obtained in two ways. In the presence of air carbohydrates are oxidized to carbon dioxide and water. This process is generally called respiration. In the absence of air they are split into simpler substances of lower free-energy content than the original carbohydrates. This anaerobic fission is referred to as fermentation, or often simply as glycolysis. Both these reactions involve a loss in free energy, and from each the energy necessary for life can be obtained. The anaerobic fission of

carbohydrates by living matter is characterized by a high rate of carbohydrate destruction and by the accumulation of certain incompletely oxidized products. When air or oxygen is admitted to the system, the rate of carbohydrate destruction is much reduced, and the formation of the typical products of anaerobic metabolism is either diminished or completely suppressed. The action of oxygen in checking this high rate of loss of carbohydrate, and in suppressing or diminishing the accumulation of these products of anaerobic metabolism, is called the Pasteur effect. This action of oxygen preserves the carbohydrate food reserves of the cell from needless destruction and is thus of the greatest importance in biological economy.

As considerable confusion exists in the current literature as to the real nature of the Pasteur effect, it is necessary to explain Pasteur's original conceptions and to describe his experimental results on the effect of oxygen on carbohydrate catabolism.

Pasteur first realized that the presence of oxygen reduces the destruction of sugar by living organisms. Alcoholic fermentation by yeast was the first example of anaerobic fission of carbohydrate to be discovered. Pasteur (1861, 1875) showed that the weight of sugar destroyed in fermentation is completely out of proportion to the weight of yeast substance formed. Pasteur defined the "power of a ferment" as the ratio of sugar destroyed to yeast substance formed. According to Pasteur it is by the high value of this ratio in certain instances that fermentation is characterized. In the presence of air this high carbohydrate destruction, in proportion to the weight of yeast formed, is not so marked. In fact Pasteur was able to vary the ratio of sugar destroyed to yeast substance formed from 176/1, in the complete absence of air, to 4/1 under markedly aerated conditions. Under conditions of high aeration the power of fermentation is thus at its feeblest. In fact oxygen reduces the power of fermentation. Pasteur says of fully aerated yeast, "*elle n'est plus ferment*".

Pasteur did not prove conclusively that the rate of sugar destruction in a given time is less in the presence than in the absence of air, although he maintained this to be the case. This was first proved in the case of yeast by Meyerhof (1925).

Pasteur of course also realized, as did others in his time, that oxygen prevents the accumulation of the products of anaerobic fission of carbohydrates. It was first shown by Lechartier & Bellamy (1869) at the instigation of Pasteur that certain fruits produce alcohol and carbon dioxide in the absence of air. In the presence of air of course this alcohol is not formed. Pasteur (1875) suggested that the suppression of alcoholic fermentation under aerobic conditions is either due to the fact that the alcohol is not formed at all in air, or that if it is formed it is immediately oxidized.

Accordingly we define the Pasteur effect as the action of oxygen in diminishing carbohydrate destruction and in suppressing or decreasing the accumulation of the products of anaerobic metabolism.

Our definition of the Pasteur effect involves two characteristics. It is evident that the first characteristic of necessity involves the second, since if there is decreased catabolism of carbohydrate, less cleavage products must be formed. However, decrease in the formation of anaerobic cleavage products does not necessitate decreased carbohydrate catabolism. Indeed the aerobic destruction of carbohydrate might be quantitatively greater than the anaerobic, even though less

anaerobic cleavage products accumulate in oxygen. It is thus merely redundant to add the second quality in defining the Pasteur effect, although as often loosely defined it is this quality which is mainly accentuated.

In this review the Pasteur effect will be considered from the standpoint of the definition given above. In the past it has been frequent for the mere action of oxygen in suppressing the accumulation of anaerobic fission products to be called the Pasteur effect in disregard for the fact that Pasteur's work was also concerned with the action of oxygen on the actual destruction of sugar. Further, the Pasteur effect has often been confused with one of the explanations which have been advanced for its mechanism. Thus the oxidative resynthesis of carbohydrate from the initial products of anaerobic fission, suggested by Wortmann and by Meyerhof, has been called the Pasteur-Meyerhof reaction. This reaction may sometimes take place, but since it is certain that it does not occur in some tissues where the Pasteur effect is in operation, it should in no wise be confused with the actual action of oxygen in diminishing carbohydrate catabolism. The earlier work will be considered where necessary, but, for other earlier references, the reader is referred to the excellent review by Lohmann (1933) on the Pasteur-Meyerhof reaction, in which the description of this theory of Meyerhof takes a pre-eminent place.

II. METHODS OF DEMONSTRATING AND MEASURING THE PASTEUR EFFECT

(1) DIRECT MEASUREMENT OF THE RATE OF CARBOHYDRATE DESTRUCTION

The fundamental method of demonstrating the Pasteur effect in any tissue is to show that the rate of carbohydrate destruction in that tissue is less in the presence of oxygen than under anaerobic conditions. This direct method has but rarely been employed. Pasteur's original experiments were pursued along these lines but were not completely conclusive. Meyerhof (1920 *b*) showed that the rate of loss of muscle glycogen is less in oxygen than in anaerobiosis. Negelein (1925 *a*) showed that the destruction of glucose in the red blood corpuscles of both the goose and the rabbit is less under normal conditions of aeration than in the presence of cyanide. He considered that in the presence of cyanide biological anaerobiosis is realized. Dixon (1936 *b*) directly compared the rate of destruction of glucose by slices of cerebral cortex of the rabbit in oxygen with that in nitrogen. He found that in the presence of oxygen the rate of carbohydrate destruction is less than 50 per cent of that obtaining under anaerobic conditions. Dixon used the symbols $Q_{C_6}^{O_2}$ and $Q_{C_6}^{N_2}$ to represent the rates of loss of hexose in oxygen and in nitrogen respectively. The symbol Q_C denotes the number of $\mu\text{l.}^1$ of hexose expressed as a gas at N.T.P.

¹ 1 millimol of any substance, if it could exist in the gaseous state, would occupy 22,400 $\mu\text{l.}$ at N.T.P. It is convenient to express on this volumetric scale the amounts of substances taking part in tissue metabolism. Firstly, by this method one can more easily compare the relative numbers of molecules of different substances, which participate in metabolic reactions, than if the amount of metabolite were measured in terms of weight, since the volume of a gas at N.T.P. is proportional to the number of molecules which it contains. Secondly, the numerical values of the quotients expressed on this scale are of a convenient order. This convention was first introduced by Warburg (see below) for expressing the rate of formation of lactic acid.

destroyed by 1 mg. dry weight of tissue per hour. In this way the rate of sugar destruction is described numerically in figures of a convenient order. When $Q_{C_6}^{O_2}$ is numerically less than $Q_{C_6}^{N_2}$ the Pasteur effect is in operation. Thus, in cerebral cortex of the rabbit $Q_{C_6}^{O_2}$ is of the order of -3 , while $Q_{C_6}^{N_2}$ is of the order of -8 (Dixon, 1936*b*). The Pasteur effect has also been directly demonstrated in the metabolism of *B. acidificans longissimus* (Meyerhof and Finkle, 1926)¹ and of *C. diphtheriae* (Passmore, unpublished), and a very slight Pasteur effect has been directly shown to occur in tumour (Negelein, 1925*a*).

(2) CALCULATION OF THE RATE OF CARBOHYDRATE DESTRUCTION

From the rates of lactic acid production and of respiration the rate of carbohydrate breakdown can be calculated in animal tissues which produce lactic acid as their sole glycolytic product and in which the total aerobic respiration is due to carbohydrate oxidation. Thus Dixon (1936*b*) showed that the quotients $Q_{C_6}^{N_2}$ and $Q_{C_6}^{O_2}$ can be calculated in cerebral cortex from the quotients Q_M and Q_{O_2} used by Warburg (1925) and Negelein (1925*b*) to represent glycolysis and respiration respectively. These quotients are defined as follows:

$Q_M^{O_2} = \mu\text{l. of lactic acid expressed as a gas at N.T.P. evolved per mg. dry weight of tissue per hour in oxygen.}$

$Q_M^{N_2} = \mu\text{l. of lactic acid expressed as a gas at N.T.P. evolved per mg. dry weight of tissue per hour in nitrogen.}$

$Q_{O_2} = \mu\text{l. of oxygen absorbed per mg. dry weight of tissue per hour.}$

Q_M is positive when lactic acid is being produced. Q_{O_2} is negative so as to indicate that oxygen is absorbed.

According to this same convention Q_{C_6} is negative so as to indicate that carbohydrate is being lost.

From the equations $C_6H_{12}O_6 = 2C_3H_6O_3$
lactic acid

and $C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O$,

which represent respectively the glycolytic and respiratory processes occurring in brain, it follows that

$$Q_{C_6}^{N_2} = -\frac{Q_M^{N_2}}{2}$$

and

$$Q_{C_6}^{O_2} = -\frac{Q_M^{O_2}}{2} + \frac{Q_{O_2}}{6}.$$

Thus $Q_{C_6}^{O_2}$ and $Q_{C_6}^{N_2}$ can be calculated from measurements of respiration and glycolysis. In this way it was also found (Dixon, 1936*b*) that $Q_{C_6}^{O_2}$ is numerically less than $Q_{C_6}^{N_2}$ in cerebral cortex, i.e. the Pasteur effect is operating. The absolute value of $Q_{C_6}^{O_2}$ can only thus be calculated in tissues in which carbohydrate is the sole respiratory fuel. In other tissues $Q_{C_6}^{O_2}$ as above calculated would be an upper limit of the rate of carbohydrate destruction in oxygen, since other substances are also being respired.

¹ The author is indebted to Dr R. Passmore for having called his attention to this work.

(3) THE MEYERHOF QUOTIENT AS APPLIED TO ANIMAL TISSUES

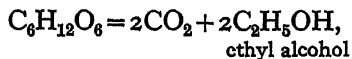
The method hitherto most generally used in demonstrating the Pasteur effect is by measurement of the Meyerhof quotient. This again is only an indirect method. The Meyerhof quotient was defined by Warburg *et al.* (1924) as

$$\frac{Q_M^{N_2} - Q_M^{O_2}}{-Q_{O_2}}$$

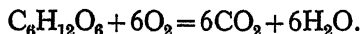
It is in fact a measure of the reduction in the rate of lactic acid production caused by oxygen, divided by the rate of respiration. The Meyerhof quotient is generally between 1 and 2. From the normal value of this quotient it may be inferred that the rate of carbohydrate destruction in oxygen is less than that in nitrogen. For, if the carbohydrate destruction in oxygen were as great as that in nitrogen, the lactic acid formation in oxygen would be less than that formed in nitrogen merely by an amount equivalent to the lactic acid (or carbohydrate) oxidized. But, since 1 molecule of lactic acid is completely oxidized by 3 molecules of oxygen, the decrease in lactic acid production thus occasioned would equal one-third of the respiration. Hence the difference between $Q_M^{N_2}$ and $Q_M^{O_2}$ would equal one-third of Q_{O_2} if the carbohydrate destruction were as great in oxygen as in nitrogen. Here the Meyerhof quotient would equal one-third. It is clear that if the difference between $Q_M^{N_2}$ and $Q_M^{O_2}$ were greater than one-third of Q_{O_2} , then the rate of carbohydrate destruction in oxygen cannot be as great as that in nitrogen. In other words, if the Meyerhof quotient is greater than one-third, the Pasteur effect is working. The work of Warburg and his colleagues has in this way demonstrated the Pasteur effect in numerous animal tissues (see Warburg, 1926*b*). Warburg *et al.* (1924) were in fact the first to emphasize the general biological significance of the Pasteur effect.

(4) THE MEYERHOF QUOTIENT AS APPLIED TO PLANT TISSUES

In many plant tissues glycolysis (i.e. alcoholic fermentation or the so-called "anaerobic respiration") is represented by the equation



while aerobic carbohydrate respiration is represented by the equation



Meyerhof (1925) introduced the following symbols to denote the metabolic quotients of yeast metabolism:

$Q_{CO_2}^{N_2}$ = μ l. of CO_2 of fermentation produced per mg. dry weight per hour in nitrogen,

¹ We place a negative sign before Q_{O_2} so that the whole quotient becomes positive.

$Q_{CO_2}^O = \mu l.$ of CO_2 of fermentation produced per mg. dry weight per hour in oxygen

(in oxygen an amount of carbon dioxide equal to the oxygen uptake is assumed to be produced by respiration),

Q_{O_2} is used in the same sense as described above.

Meyerhof found the rate of formation of carbon dioxide of fermentation to be reduced in the presence of oxygen. The term Meyerhof quotient in such plant tissues is used to denote

$$\frac{Q_{CO_2}^N - Q_{CO_2}^O}{-Q_{O_2}}$$

This quotient measures the decrease in carbon dioxide of fermentation divided by the oxygen uptake. It is clear from the above equations that this quotient

$$= \frac{\text{decrease caused by oxygen in sugar fermented} \times 2}{\text{sugar oxidized} \times 6}$$

or the Meyerhof quotient

$$= \frac{\text{decrease caused by oxygen in sugar fermented}}{\text{sugar oxidized}} \times \frac{1}{3}.$$

Thus, when the Meyerhof quotient equals one-third, the reduction effected by the presence of oxygen in the quantity of sugar fermented equals the amount of the sugar oxidized. Hence, when the Meyerhof quotient equals one-third, the rate of destruction of carbohydrate is as great in oxygen as in nitrogen. If the Meyerhof quotient is greater than one-third, then the decrease caused by oxygen in the quantity of sugar fermented is greater than the quantity of sugar oxidized, and the carbohydrate destruction is less in oxygen than in nitrogen. The Pasteur effect is here in operation. Meyerhof found the Meyerhof quotient to vary in yeast between 1 and 2. In this way Meyerhof (1925) demonstrated the Pasteur effect in yeast. Windisch (1932), however, maintained that the Pasteur effect does not work in large-scale experiments on yeast. Hoogerheide (1935 *a*) on the other hand in a careful series of experiments has shown that Windisch's results were due to deficient aeration. Hoogerheide has amply confirmed Meyerhof's micro-determinations by finding the carbon dioxide of fermentation in the presence of oxygen to be much decreased also in large-scale experiments. The values for the Meyerhof quotient calculated from his experiments are well above one-third. Genevois (1927 *a, b*) by measurements of the Meyerhof quotient has demonstrated the Pasteur effect in various green plants and seedlings. Here the Pasteur effect is not so marked as in yeast, since the Meyerhof quotient is lower, though still substantially greater than one-third.

(5) THE I/N RATIO IN PLANTS

In most higher plants in air respiration alone occurs; fermentation only occurs in anaerobiosis. The amount of carbon dioxide produced in anaerobic fermentation or "intramolecular respiration" is called I , while the carbon dioxide produced in

normal aerobic respiration is called N (see Kostytschew, 1924). The ratio I/N was first measured by Wortmann (1880). The I/N ratio gives a measure of the extent of the Pasteur effect in plants. If the respiratory destruction of sugar were equal to the fermentative destruction, then three times as much carbon dioxide should be produced in respiration as in fermentation. This follows from the fact that 1 sugar molecule produces 2 molecules of carbon dioxide by fermentation and 6 molecules of carbon dioxide in respiration. Hence, if $I/N = \frac{1}{3}$, then the sugar destruction in oxygen is as great as that in nitrogen. If $I/N > \frac{1}{3}$, then the carbohydrate destruction is less in oxygen than in anaerobiosis. Wortmann (1880) found in pea seedlings that $I/N = 1$ and thus was the first to demonstrate the Pasteur effect in higher plants. This method has been much used in the past in other plant tissues (see Kostytschew, 1924) and it is clear that the Pasteur effect is frequently not so marked as in the case recorded by Wortmann. Recently this ratio has again been the subject of much research. Blackman & Parija (1928) have thus demonstrated the Pasteur effect in apples, obtaining values of I/N even greater than unity (Blackman & Parija called I and N respectively NR and OR). Leach (1936) has shown, however, that in certain seedlings I/N is only just greater than $\frac{1}{3}$; here presumably the Pasteur effect is scarcely in operation (see also Leach & Dent, 1934).

Both this method and the method involving measurement of the Meyerhof quotient in plant tissues do not amount to rigid proof that the Pasteur effect is occurring. Both methods assume that the amount of alcohol formed in plant tissues in anaerobiosis is equivalent to the carbon dioxide of fermentation. This is very nearly the case in yeast fermentation and thus Meyerhof's work is not open to doubt. However, in the higher plants the equation of alcoholic fermentation is but rarely followed stoichiometrically and frequently the alcohol produced falls far short of equivalence to the carbon dioxide (Kostytschew, 1913). It would appear really important that the work on plants should be substantiated by direct measurement of loss of carbohydrate as has been done in the case of muscle, brain and bacteria.

III. CONDITIONS GOVERNING THE OPERATION OF THE PASTEUR EFFECT

(1) DEPENDENCE ON RELATIVE MAGNITUDES OF RESPIRATION AND GLYCOLYSIS

The property by which oxygen impedes carbohydrate catabolism and suppresses glycolysis is apparently dependent on oxygen being absorbed by the cell. Pasteur (1861) first suggested that glycolysis (fermentation) is impeded by the actual absorption of oxygen in cellular respiration. According to this view the effect of respiration in curbing glycolysis should be proportional to the magnitude of the respiration, as indeed has been indicated by the work of Warburg (1926*b*) and others in demonstrating that the Meyerhof quotient is a fairly constant entity. Further, if cell respiration were eliminated glycolysis and carbohydrate destruction should proceed unimpeded by the presence of oxygen. This was first shown by Meyerhof (1920*b*) who found that the addition of cyanide, which inhibits respiration, increases the aerobic glycolysis in chopped muscle. Later Warburg *et al.* (1924) showed that

in the presence of cyanide the glycolysis of tumour and embryo approaches the high anaerobic level.

It is thus clear that conditions which inhibit respiration abolish, or at any rate diminish, the magnitude of the Pasteur effect. Conversely, conditions which increase the glycolysis without causing a corresponding increase in respiration make the Pasteur effect appear insignificant, so that aerobic glycolysis becomes apparent. This lack of balance between respiration and glycolysis occurs naturally in some tissues and can be effected artificially. We will now consider the examples of aerobic glycolysis caused by this lack of balance between the magnitudes of respiration and glycolysis and the relative exiguity of the Pasteur effect. The aerobic glycolysis which we will now discuss is not caused by reduction in the Meyerhof quotient. The quantitative effect of respiration on glycolysis is not reduced, but the relative magnitude of respiration to glycolysis is so low as to render the effect of respiration incomplete or negligible.

Among plants aerobic glycolysis is characteristic of the normal metabolism of certain fungi. Among the fungi we encounter all stages between brewer's yeast, where respiration is very small and where the high fermentation proceeds almost unimpaired in the presence of oxygen, and certain wild yeasts and moulds where no aerobic glycolysis occurs. Meyerhof (1925) first demonstrated that a relation exists between the decrease caused by oxygen in the fermentative activity and the magnitude of respiration. If the anaerobic fermentation is relatively high, then the respiration is inadequate to suppress it, and so aerobic glycolysis occurs. Brewer's yeast is at one end of the scale with high fermentation and low respiration. It shows high aerobic glycolysis. *Torulae*, wild yeasts and moulds are at the other end of the scale with low fermentative activity, high respiration and no aerobic glycolysis. Baker's yeast occupies an intermediate position. Further, these types of metabolism can be altered by conditions of culture.

In most normal animal cells aerobic glycolysis does not occur. Abnormal conditions can, however, by inhibiting respiration, cause the assumption of aerobic glycolysis. Thus Meyerhof (1920*b*) and Warburg *et al.* (1924) have caused aerobic lactic acid accumulation in muscle and embryo by treatment with cyanide. Further, Warburg *et al.* showed that when embryonic cells are deprived of oxygen for a certain space of time the respiration becomes so reduced that aerobic glycolysis occurs. Similar aerobic glycolysis due to irreversible loss in respiration is shown by white bone marrow cells on incubation in Ringer's solution (Fujita, 1928).

The metabolism of tumour cells presents a most remarkable example of aerobic glycolysis due to deficient respiration. Warburg *et al.* showed that the Meyerhof quotient is normal in many tumour cells so that the aerobic glycolysis is merely due to deficient respiration and not to diminished efficiency of respiration in causing the Pasteur effect. However, Crabtree (1929) has shown that in some cases in tumour the respiration is of high order and yet only slightly diminishes glycolysis; he observed Meyerhof quotients as low as 0.2. It would appear that here the aerobic glycolysis is due not to deficiency in the magnitude of respiration, but to the inefficiency of this respiration in suppressing glycolysis. However, in Crabtree's experiments this high

respiration was probably not due to carbohydrate oxidation, since the addition of glucose actually reduced the respiration. So the magnitude of *carbohydrate* respiration may indeed be too low to reduce glycolysis. That carbohydrate respiration alone is responsible for the Pasteur effect has been suggested by Dickens & Šimer (1930). These workers showed that lactate, which increases the respiratory quotient of liver and testis, does not increase the respiratory quotient of tumours, while the respiratory quotient of all these tissues is increased by pyruvate. Dickens & Šimer suggested that the oxidation of lactate to pyruvate is deficient in tumours and that it is this defect in carbohydrate oxidation which leads to the aerobic accumulation of lactic acid in malignant tissue. According to Elliott *et al.* (1935) it is the oxidation of succinate which is deficient in tumours. However, Dickens & Weil-Malherbe (1935) have demonstrated that succinate is readily oxidized in the Jensen rat sarcoma. The aerobic glycolysis of tumours is thus probably due to a high glycolytic rate with a relatively low carbohydrate respiration. Why the carbohydrate respiration is defective is not at present certain.

As regards normal animal cells a slight aerobic glycolysis due apparently to high glycolytic power is observed in brain and testis (Warburg *et al.* 1924). A more marked aerobic glycolysis is seen in embryonic lens (Fujita, 1928). Aerobic glycolysis also occurs in red blood corpuscles of mammals but not in the nucleated red blood corpuscles of birds (Negelein, 1925 *a*). The aerobic glycolysis was attributed by Negelein to deficient respiration following loss of the nucleus in adult mammalian erythrocytes. Warburg *et al.* (1924) showed that normal mammalian retina has a relatively enormous aerobic glycolysis even when the greatest care is exercised to avoid injury to the tissue. Kubowitz (1929) found that frog's retina if undamaged does not show aerobic glycolysis except when the temperature is raised. Kubowitz believed it impossible to maintain isolated mammalian retina at mammalian body temperature *in vitro* without cellular damage occurring. This damage might consist in injury to the mechanism whereby respiration suppresses glycolysis or simply in inhibition of respiration; in either case aerobic glycolysis would be produced. The first possibility is rendered improbable by the finding of Warburg *et al.* that the Meyerhof quotient of mammalian retina is normal in value. Krebs (1927) has found the same for chick retina. In the second place, it is unlikely that respiration itself has been artificially reduced, since Krebs found that the respiration of chick retina declines and that the aerobic glycolysis increases progressively throughout development until, in the retina of the adult hen, there is *no* respiration and the aerobic lactic acid production equals the anaerobic. This work of Krebs makes it extremely probable that the aerobic glycolysis of retina is merely due to the relatively high value of glycolysis in relation to the magnitude of the respiration obtaining in this tissue. Aerobic glycolysis, due to this same cause, occurs in normal kidney medulla (György *et al.* 1928, Dickens and Weil-Malherbe, 1936).

We will now consider the conditions where aerobic glycolysis is caused by a sudden increase in the magnitude of glycolysis which renders the respiration relatively insufficient to suppress glycolysis. Firstly, in muscular activity the rate of glycolysis is so increased that aerobic lactic acid accumulation occurs (Fletcher &

Hopkins, 1907). The same effect is caused by chopping the muscle (Meyerhof, 1920*b*), or by adding excess of potassium ions (Hegnauer *et al.* 1934). Further it has been found (Dixon, 1936*c*) that marked aerobic glycolysis occurs in rabbit's brain cortex at 45° C. At this high temperature the rate of anaerobic glycolysis is greatly increased and although the respiration is also raised it is no longer sufficient to suppress glycolysis. The Meyerhof quotient is not subnormal.

(2) SPECIFIC INHIBITION OF THE PASTEUR EFFECT

Warburg (1926*a*, 1929) suggested that in certain cases the Pasteur effect could be inhibited alone without a fall in respiration. He considered the Pasteur effect to be inhibited where the efficiency of respiration in curbing glycolysis (as measured by the Meyerhof quotient) is subnormal. Warburg thus regarded aerobic glycolysis as due to two causes, firstly, to deficiency in respiration, and secondly, to inefficiency of respiration in suppressing glycolysis and causing the Pasteur effect. Later Dickens (1934*a*) delimited aerobic glycolysis due to specific inhibition of the Pasteur effect. Here the Meyerhof quotient is subnormal but the respiration is undiminished.¹ In more general terms the Pasteur effect is sometimes deficient or abolished under conditions where respiration is neither inhibited nor of low magnitude. In fact the normal link between respiration and glycolysis whereby the former retards the latter has become dislocated. We will now describe the conditions under which the Pasteur effect is in this manner deficient or inhibited.

(a) *Inhibition by cellular damage*

Firstly, certain conditions which apparently damage the cell irreversibly cause this specific inhibition of the Pasteur effect. Thus Negelein (1925*a*) found that, when embryonic tissue is allowed to remain for some time in Ringer's solution, aerobic glycolysis develops without there occurring any fall in respiration. This effect is irreversible and it does not occur when the tissue is kept in serum. Kubowitz (1929) found that abnormally high temperatures reduce the Meyerhof quotient to zero when the respiration is only 70 per cent inhibited. This, however, is not a case of specific inhibition of the Pasteur effect since the respiration is inhibited. Nakashima (1929), however, showed that the Meyerhof quotient of fish retina is reduced similarly by high temperatures and here there is actually a rise in respiration. In view of the similar effect described by Dixon (1936*c*) in brain cortex, where the high anaerobic glycolysis falls very rapidly at high temperatures, it is not certain that the Meyerhof quotient in fish retina is indeed subnormal if it were measured at the commencement of the experiment. Dixon found a very rapid fall in anaerobic glycolysis of brain at 45° C., though the aerobic glycolysis rises slightly at first. Perhaps the aerobic glycolysis in fish retina at high temperatures is thus not caused

¹ Actually Dickens suggested that it might be impossible to inhibit the Pasteur effect without inhibiting the oxidation of carbohydrates and that, where respiration is not diminished, substances other than carbohydrate are being oxidized to an increased extent. It has, however, now been established by the work of Ashford and Dixon (1935) and of Dixon (1936*b*) (see below) that the Pasteur effect can be completely abolished when carbohydrate respiration is undiminished. Dickens (1935) also arrived at a similar conclusion.

by specific inhibition and is merely due to an abnormally high glycolytic rate with relative insufficiency of respiration, as is the case in cerebral cortex.

Bumm *et al.* (1932) found that reduction in oxygen pressure does not inhibit the respiration of intestinal mucosa but inhibits the Pasteur effect. It is unfortunate that their measurements of respiration were carried out in phosphate Ringer while the glycolysis was measured in bicarbonate Ringer. The work of Laser (1935) has demonstrated striking differences in the metabolic behaviour of retina when immersed in these different media. Further, Leiner (1935) found that in embryonic blood corpuscles reduction in oxygen-tension does not inhibit the Pasteur effect so long as respiration is not reduced.

Dickens (1934*a*) found that pretreatment of testis and tumour cells with phenylhydrazine causes an increase in aerobic glycolysis without a fall in respiration. This effect is irreversible.

Stier & Stannard (1936) have described another case of cellular damage causing specific inhibition of the Pasteur effect. Baker's yeast, when not supplied with sugar, respire its own glycogen without any aerobic glycolysis occurring. On grinding the cells, however, Stier & Stannard found that, although the respiration is actually increased, aerobic alcoholic fermentation commences.

(b) Inhibition by certain substances added in low concentrations to the environmental medium

Secondly, specific inhibition of the Pasteur effect can be effected in tissues by the addition in low concentrations of certain abnormal substances to the medium bathing the cells. Many of these substances simultaneously cause a rise in respiration but most at a higher concentration also inhibit respiration.

(1) Warburg (1926*a*) showed that in the presence of $10^{-3} M$ ethyl isocyanide the aerobic glycolysis of both normal and malignant tissue is raised to the anaerobic level. The respiration is unchanged.

(2) Genevois (1927*b*) showed that cyanide in a concentration of 10^{-3} to $10^{-5} M$ causes aerobic glycolysis in sweet-pea seedlings without causing any fall in the respiration, which is even sometimes slightly increased. Laser (1935) has since shown that low concentrations of cyanide can increase the aerobic glycolysis of retina to the anaerobic level without fall in respiration.

(3) Krah (1930) showed that many heavy metal reagents increase aerobic glycolysis in tumours without decreasing respiration. Of these most (e.g. 5-nitro 4-chlor 2-amido phenol) cause an increase in respiration. One of these reagents, however (1-amido 2-naphthol 6-sulphonic acid), scarcely affects the respiration.

(4) Bumm & Appel (1932) quote Mendel as having shown that guanidine increases the aerobic glycolysis of tumours without inhibiting respiration. They maintain that arginine also shows this effect.

(5) Bumm & Appel (1932) showed that reduced glutathione at a concentration of $10^{-3} M$ increases aerobic glycolysis in tumours without any fall in respiration. Quastel & Wheatley (1932*b*) have also shown that reduced glutathione can increase aerobic glycolysis in baker's yeast without fall in respiration.

(6) Dodds & Greville (1934) showed that 4-6-dinitro *o*-cresol increases both respiration and aerobic glycolysis in thin slices of Jensen rat sarcoma. Earlier (1933) they showed that this reagent increases the oxygen uptake of kidney. Recently Krah1 & Clowes (1935) have shown that dihalo phenols have a similar action on respiration. It is interesting that the dinitro phenols are strongly calorigenic in action when injected into the whole organism (Dodds & Pope, 1933), whereas the halogenated phenols are apparently without action (Krah1 & Clowes). These effects are apparently distinct from the increases in respiration caused by reversible dye-stuffs (Barron & Harrop, 1928; Dickens, 1934*b*) since Greville & Stern (1935) have shown that the dinitro phenols are not reversibly oxidized and reduced under these conditions and also since the dihalo phenols have apparently a similar action to the dinitro derivatives.

(7) Dickens (1935) showed that phenosafranine at a concentration of $10^{-5}M$ increases aerobic glycolysis in brain and tumour without depressing respiration. More recently Dickens (1936*a*) has shown that the tumour producing 2-*p*-amino styryl 6-*p*-acetylamino benzoylamino quinoline methoacetate increases respiration and raises the aerobic glycolysis to the anaerobic level (the anaerobic glycolysis being also raised).

(8) Weil-Malherbe (1935) has found that glutamate and maleate inhibit the Pasteur effect in brain without reducing respiration. Glutamate also decreases the anaerobic glycolysis and raises the aerobic glycolysis to the anaerobic level. The effect with glutamate is only observed in brain tissue. Both effects are abolished by the simultaneous addition of pyruvate.

(c) Inhibition due to lack of ionic balance in the environmental medium

In the third place, lack of balance in the ionic constituents of the medium bathing the cells can bring about specific inhibition of the Pasteur effect in brain cortex. Thus Ashford & Dixon (1935) found that the addition of $M/10$ KCl to the Ringer, in which mammalian brain cortex slices are suspended, causes the assumption of marked aerobic glycolysis and an increase in respiration.¹ This effect is reversible. Further it can also be caused by rubidium and caesium chlorides (Dixon & Holmes, 1935; Dickens & Greville, 1935), though sodium chloride in similar concentration is almost without effect (Ashford & Dixon, 1935; Dickens & Greville, 1935). Unpublished observations show that this effect is seen equally well with $M/20$ KCl, though at $M/100$ only a small effect is demonstrable. That the Pasteur effect really is inhibited by the addition of potassium ions has been proved by Dixon, who found (1936*b*) that the aerobic rate of destruction of carbohydrate in the presence of excess of potassium ions is raised to the normal anaerobic level. In the absence of oxygen it has been found (unpublished observation) that the destruction of carbohydrate is markedly inhibited by potassium ions. We thus know that, in the presence of added potassium ions, oxygen no longer diminishes the rate of destruction of carbohydrate. It is therefore clear that the Pasteur effect can be

¹ I wish to thank Dr Laser for suggesting that respiration might also be increased under these conditions owing to stimulation of metabolism.

eliminated by conditions which do not diminish carbohydrate respiration and also that the Pasteur effect is not a necessary consequence of carbohydrate oxidation. The anaerobic glycolysis is not increased by potassium but is markedly inhibited (Ashford & Dixon); this effect is irreversible. The difference between the aerobic and the anaerobic potassium effects is probably due to the respiration under aerobic conditions preventing potassium ions from damaging the cells (see Dickens and Greville, 1935). Inhibition of the respiration by cyanide causes a fall in the high aerobic glycolysis reducing it to the low anaerobic value (Dixon, unpublished).

The potassium effect was originally shown where glucose is the substrate supplied to the cells; it is not seen in the absence of substrate (Dixon, 1935; Dickens & Greville, 1935). Recently Dixon (unpublished) has observed an effect with mannose similar to that shown by glucose. With fructose, lactate and pyruvate (substrates which in brain cannot give rise to lactic acid) there is also an increase in respiration on the addition of potassium chloride (Dixon & Holmes, 1935; Dickens & Greville, 1935). This precludes the possibility suggested by Chang & Tai (1936) that the potassium effect on respiration in brain is caused by the increase in lactic acid formation, although this may be the case in muscle (see Hegnauer *et al.* 1934).

The potassium effect on the glycolysis can be abolished by the simultaneous addition of calcium ions (Dixon & Holmes, 1935). Unpublished results of the author show that this is also the case with the respiration when it is measured simultaneously. Further a similar inhibition of the Pasteur effect can be effected by the removal of both calcium and potassium ions from the Ringer's solution bathing the cells (Dickens & Greville, 1935).

Recently Chang & Tai (1936) have made a large number of experiments on the respiration of the central nervous system and peripheral nerve of the toad. They found that both the respiration of the brain and spinal cord is increased by potassium and lowered by calcium. The potassium effect does not occur in peripheral nerve, where potassium actually lowers the respiration. Chang & Tai believe the effect to be confined to grey matter which is abundant in spinal cord as well as in brain. Chang & Tai also obtained a similar effect by immobilizing calcium by the addition of citrate—a rise in respiration occurs, as indeed was also found in rat brain by Dickens & Greville (1935) on removal of calcium from the Ringer solution surrounding the cells.

These effects of ions in specifically inhibiting the Pasteur effect have only been observed in nervous tissue. In muscle aerobic glycolysis with rise in respiration can be caused by addition of potassium under aerobic conditions (Hegnauer *et al.* 1934), but here the anaerobic glycolysis is also increased. It is probable that in muscle the Pasteur effect is not specifically inhibited; it would seem that the aerobic glycolysis is merely due to the abnormally high glycolytic rate relative to the respiration. An increase in respiration is also seen on the addition of potassium ions to the medium surrounding the cilia of the gill plates of *Mytilus* (Gray, 1924). Here as in muscle the effect is associated with increased contraction or motion. It is uncertain whether this is a case of specific inhibition.

Dickens & Greville (1935) showed that there is no potassium effect in mammalian

tumour, kidney, testis and yolk sac, while Dixon, Needham & Nowinski (unpublished) have demonstrated its absence in chick embryo. The effect of lack of ionic balance in causing specific inhibition is probably confined to nervous tissue. This may be due to the fact that the metabolism of neurones is superficially localized in the dendrites of the nerve cells as has been indicated by the work of Holmes (1932). It was suggested by Dixon (1936*a*) that only in nervous tissue can changes in ionic balance of the external medium affect the active portions of the cells owing to the superficial localization of metabolic activity. Unpublished observations show that the respiration of brain cortex in the presence of succinate and of glutamate is not augmented by the addition of potassium. In this way the oxidation of succinate and glutamate differs from that of glucose, lactate, etc. The different location of these systems in the cell may account for these differences in their behaviour.

(*d*) *Natural specific inhibition or absence of the Pasteur effect*

Lastly, in certain cells with an otherwise normal carbohydrate respiration oxygen has little effect in suppressing carbohydrate catabolism. Here the link between respiration and glycolysis appears to be absent. Thus in all plants where the I/N ratio is as low or lower than 0.33 the Pasteur effect is not operating. At this value of I/N the rate of carbohydrate destruction in oxygen is as great as that in nitrogen. Cases of such low I/N ratio have been reported in higher plants (see Kostytschew, 1924; Boysen-Jensen, 1930). In some cases, e.g. that of the buckwheat (Leach, 1936), the I/N ratio is just above 0.33; here only a slight Pasteur effect is operating. In *Chlorella* (Genevois, 1927*a*) it would appear from measurements of respiration and fermentation that there is no Pasteur effect. Genevois's later results (1927*b*) would indicate that the Pasteur effect slowly disappears in the germinating sweet-pea seedling—the adult tissue having hardly any Pasteur effect.

In spite of the frequent absence of the Pasteur effect in higher plants aerobic glycolysis (or fermentation) is not usually observed, since the respiration has such a high value in comparison with the fermentation that all the products of fermentation can be removed by oxidation although this oxidation does not reduce carbohydrate destruction. In some plants there is no anaerobic fermentation, here also there can be no Pasteur effect.

(3) SPECIFIC AUGMENTATION OF THE PASTEUR EFFECT

It has been shown by Laser (1934) that the addition of lactoflavin to tissue cultures which normally show some aerobic glycolysis almost completely suppresses the aerobic glycolysis, although the anaerobic glycolysis is unaffected. The Meyerhof quotient is thus increased and the effect of respiration in reducing carbohydrate catabolism is increased. The Meyerhof quotient may also be increased in baker's yeast by the addition of yeast water to the suspension (Meyerhof & Iwasaki, 1930). Further, rise in temperature increases the Meyerhof quotient in frog retina (Kubowitz, 1929).

IV. MECHANISM

In order to explain the mechanism of the Pasteur effect it is necessary to explain the action of oxygen in reducing carbohydrate catabolism. It is not enough to explain the action of oxygen in suppressing the accumulation of anaerobic fission products, since it is possible for the rate of carbohydrate destruction to be as great in oxygen as in nitrogen, although less of these fission products are accumulating in oxygen owing to their oxidative removal. The action of oxygen in lessening the accumulation of fission products does not by itself constitute the Pasteur effect, but the reduction in this accumulation which is a necessary consequence of the action of oxygen in decreasing carbohydrate catabolism is a secondary manifestation of the Pasteur effect.

Two main types of explanation have been offered for the action of oxygen in diminishing carbohydrate destruction. Firstly, it is supposed that the gross rate of carbohydrate fission is the same in oxygen as in anaerobiosis, but that respiration causes or is itself constituted by the rebuilding of part of the split products to carbohydrate. In this way the net rate of carbohydrate destruction is reduced. Secondly, it has been suggested that the gross rate of carbohydrate fission is itself reduced by the presence of oxygen. The first kind of theory involves resynthesis of carbohydrate in the presence of oxygen, the second suggests that oxygen in some way inhibits the initial destruction of carbohydrate.

Many of the theories of mechanism involve the assumption that glycolysis and carbohydrate respiration have an initial common path. However, it must be emphasized that this view, if correct, cannot of itself explain the Pasteur effect. The theory that there is an initial common stage in glycolysis and respiration was first advanced by Pflüger (1875) and supported by Pfeffer (1878). Others suppose that carbohydrates are directly oxidized and an initial glycolytic stage does not occur in their catabolism. The evidence for and against the hypothesis of Pflüger and Pfeffer as regards plant tissues has been admirably discussed by Kostytschew (1924) and by Hoogerheide (1935*a, b*). Both these authors produce considerable evidence in favour of the theory. Direct oxidation can occur, however, in certain moulds as is witnessed by the production of gluconic acid (Molliard, 1922, Müller, 1928) and of glucosone (Walker, 1932). As regards animal tissues it has long been considered that lactic acid is an intermediary in carbohydrate oxidation; evidence in favour of this view has come from the work of Fletcher & Hopkins (1907) on muscle, of Holmes (1930) and of Krebs (1931) on brain, and of Dickens & Šimer (1930) on testis. On the other hand Himwich & Fazikas (1935) have shown that nicotine inhibits the oxidation of lactic acid in brain but not that of glucose nor that of pyruvic acid. This would apparently exclude the view that glucose oxidation necessarily proceeds by way of lactic acid. Himwich's work, however, still leaves open the possibility that pyruvic acid is an intermediary in glucose oxidation. That pyruvic acid is an intermediary is rendered likely by the work of Peters and his collaborators (see Peters & Sinclair, 1933; Peters & Thompson, 1934; Thompson & Johnson, 1935) which shows that this substance accumulates in avitaminosis B₁. Weil-Malherbe (1936) has also

produced evidence that pyruvic acid is an intermediary in carbohydrate oxidation. Since pyruvic acid is probably a precursor of lactic acid it is thus justifiable to assume that respiration and glycolysis have an initial common path. Direct oxidation of carbohydrate may, however, also occur in the animal, as is instanced by the formation of gluconic acid by glucose dehydrogenase (Harrison, 1931) and of phosphohexonic acid by hexose monophosphate dehydrogenase (Warburg *et al.* 1935).¹ Further fructose can be oxidized by brain, a tissue in which it scarcely forms any lactic acid (Loebel, 1925). That direct oxidation of glucose to glucosone is the oxidation catalysed by insulin, as suggested by Hynd (1927), has been rendered improbable by the work of Dixon & Harrison (1932).

We may conclude that the hypothesis that carbohydrate oxidation and glycolysis have an initial common path is probably correct at least for a part of carbohydrate oxidation. Direct oxidation no doubt also occurs. We certainly cannot eliminate theories for the mechanism of the Pasteur effect, which assume the identity of the initial stages in the two processes, on the ground that this view is incorrect. We will therefore discuss these theories solely on their own merits, first considering those which involve resynthesis and no reduction in the gross rate of carbohydrate destruction and secondly considering those involving a direct inhibition of the initial stages of catabolism by the presence of oxygen.

(1) MEYERHOF'S THEORY FOR ANIMAL METABOLISM

Fletcher & Hopkins (1907) suggested that the lactic acid which is formed during muscular activity may be rebuilt to its precursor during oxidative recovery. Hill (1914) concluded from the small value of the heat evolved in recovery that not all the lactic acid disappearing can be oxidized, but that some is resynthesized to its precursor. Meyerhof (1920*a*) confirmed Hill's finding on the heat production and further showed that the lactic acid which is formed on stimulation is partly rebuilt² to glycogen during recovery. Meyerhof at the same time made the very important suggestion that the resting respiration qualitatively is identical with the processes occurring in activity and consists of a two-stage process of formation of lactic acid and its resynthesis to glycogen, as in contraction followed by recovery. Firstly, lactic acid is formed in oxygen with as high a velocity as in anaerobiosis. The lactic acid is then rebuilt to carbohydrate at the expense of energy obtained by the oxidation of a small amount of carbohydrate or of a small fraction of the lactic acid. The small net loss in carbohydrate observed in resting muscle in oxygen is due to this oxidation. There is a dynamic equilibrium in action whereby carbohydrate is continually being split to lactic acid and this is then resynthesized to carbohydrate. This consecutive catabolism and anabolism, in which the gross rate of carbohydrate destruction is as great as in anaerobiosis but in which the net rate of destruction is

¹ The recent work of Lipmann (1936) and of Dickens (1936, *b*) indicates that the further oxidation of phosphohexonic acid proceeds through an intermediary ketonic acid with subsequent liberation of carbon dioxide.

² Parnas & Wagner (1914) had shown that glycogen is the precursor of lactic acid in muscle.

markedly reduced, is called the Meyerhof cycle. This cycle would explain the action of oxygen in reducing the rate of carbohydrate destruction and this explanation has until recently been generally accepted.

The evidence for Meyerhof's theory will now be considered.

(a) *Evidence from synthesis of carbohydrate from lactic acid*

Meyerhof (1920*a*, *b*) and Meyerhof & Meier (1924) showed that, when lactic acid is produced in muscles as a result of electrical stimulation or asphyxia, there is an increase in glycogen on allowing the muscles to recover in oxygen. This increase corresponds roughly to the lactic acid which disappears and cannot be accounted for by oxidation. Meyerhof *et al.* (1925) showed that muscles incubated in Ringer's solution containing lactate possess more glycogen than those incubated in Ringer alone. Takane (1926) similarly showed that lactic acid increases liver glycogen. Meyerhof *et al.* (1925) also claimed to have demonstrated the formation of muscle glycogen on perfusing lactate through the hind limbs of a frog. Parnas & Baer (1912) had previously shown this using tortoise liver. Long & Horsfall (1932) showed that lactic acid injection along with glucose and insulin increases the synthesis of muscle glycogen above that occurring with glucose and insulin alone. Long and Horsfall believe that synthesis of muscle glycogen from lactic acid requires the simultaneous oxidation of carbohydrate. The synthesis of glycogen in the liver of the whole animal from injected lactic acid has been conclusively proved by the researches of Cori & Cori (1929), Himwich *et al.* (1930) and Long & Grant (1930).

(b) *Demonstration that more lactic acid disappears than can be accounted for by that oxidized. Oxidation quotient*

Meyerhof (1920*c*) called the ratio $\frac{\text{lactic acid disappearing}}{\text{lactic acid oxidized}}$ the oxidation quotient.

Wherever it is greater than unity lactic acid disappears in some other way besides complete oxidation. The missing lactic acid is supposed to be synthesized to carbohydrate. It has been assumed that the Meyerhof cycle is in evidence wherever the oxidation quotient is greater than unity. Also every independent method of demonstrating this high oxidation quotient is regarded as a confirmation of Meyerhof's theory. Further, according to Meyerhof's view,

the decrease in glycolytic fission of carbohydrate caused by oxygen
carbohydrate oxidized

$$= \frac{\text{lactic acid disappearing}}{\text{lactic acid oxidized}} = \text{oxidation quotient.}$$

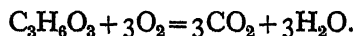
The oxidation quotient is thus supposed to be a measure of the efficiency of the Pasteur effect, which is occurring wherever this quotient is greater than unity.

The oxidation quotient was measured in frog muscle by Meyerhof (1920*c*), by Meyerhof & Meier (1924) and by Meyerhof *et al.* (1925). The lactic acid disappearing was measured directly, while that oxidized was calculated from the difference between the oxygen uptake with and without lactic acid. Values between 2 and 7 were ob-

tained. This method has been applied in some cases where synthesis of carbohydrate from lactic acid has not been demonstrated. When the quotient is greater than unity it is considered that the Meyerhof cycle obtains. For instance Long & Lupton (1923) measured the disappearance of lactic acid from the blood of men following severe exercise. The lactic acid oxidized was calculated from the excess oxygen uptake. A quotient between 4 and 10 was found. It was concluded that the Meyerhof cycle is occurring. Hartree & Hill (1922*a, b*) calculated the relative amounts of lactic acid oxidized and resynthesized in muscle during recovery on the assumption that these two reactions are responsible for the whole of the heat evolved during recovery. Their results also substantiate the Meyerhof cycle in that apparently lactic acid is both resynthesized and oxidized. Hill *et al.* (1922) calculated the oxidation quotient in still another way using the animal as a whole. Owing to the disappearance of lactic acid from the blood, free base is liberated and there is a corresponding retention of carbon dioxide. This retention of carbon dioxide is measured by respiratory analysis and this was taken as equivalent to the lactic acid disappearing. The lactic acid oxidized was calculated from the excess oxygen uptake. An oxidation quotient of 4.2 was obtained.

(c) *Evidence from the Meyerhof quotient*

Warburg *et al.* (1924) measured the Meyerhof quotient, which they defined (see above) as $\frac{\text{lactic acid formed in nitrogen} - \text{lactic acid formed in oxygen}}{\text{oxygen uptake}}$, in a number of different tissues. In general the quotient is between 1 and 2. If Meyerhof's theory is correct then the difference between the lactic acid formed in oxygen and nitrogen equals the amount of lactic acid which has been removed by oxidation and re-synthesis. Further, on Meyerhof's theory it is assumed in general that carbohydrate is converted into lactic acid prior to oxidation and that lactic acid is the substance oxidized. Lactic acid is oxidized according to the equation



Hence the lactic acid oxidized is equivalent to a third of the oxygen uptake. It follows that the Meyerhof quotient

$$\begin{aligned} &= \frac{\text{lactic acid formed in nitrogen} - \text{lactic acid formed in oxygen}}{\text{oxygen uptake}} \\ &= \frac{\text{lactic acid which disappears in oxygen}}{3 \times \text{lactic acid oxidized}} \\ &= \frac{1}{3} \times \text{oxidation quotient.} \end{aligned}$$

Thus the Meyerhof quotient $= \frac{1}{3} \times$ oxidation quotient. By measurement of the Meyerhof quotient one can thus calculate the oxidation quotient provided Meyerhof's theory is correct. The Meyerhof quotient is generally of the order of one-third of the oxidation quotient as the theory requires. It has been assumed that Meyerhof's theory of the Pasteur effect is applicable to any tissue where the Meyerhof quotient is between 1 and 3 (i.e. the oxidation quotient is between 3 and 9). In this way

Warburg *et al.* (1924) have shown Meyerhof's theory to be applicable in brain and tumour. Meyerhof & Himwich (1924) used the same method for demonstrating the Meyerhof cycle in mammalian muscle. Similar results were obtained by Meyerhof (1920*b*) and by Boyland (1931) for chopped muscle. Actually Meyerhof and his collaborators expressed their results in terms of the oxidation quotient which was derived from the Meyerhof quotient. The oxidation quotient calculated thus from the Meyerhof quotient is also of the right order in nerve (Gerard & Meyerhof, 1927). It was assumed from these data that the Meyerhof cycle occurs in all these cases and thus provides a general explanation for the Pasteur effect.

(d) Criticism of Meyerhof's theory

The earlier experiments of Meyerhof (1920*a, b*) showing that lactic acid can be synthesized to glycogen in isolated frog muscle are apparently conclusive. However, the results of Meyerhof *et al.* (1925) demonstrating this synthesis in the perfused hind limbs of a frog could not be confirmed by Eggleton & Evans (1930). Long & Horsfall (1932) maintain that in the presence of glucose and insulin lactic acid can form glycogen in mammalian muscle *in vivo*. *In vitro* it appears unlikely that this synthesis can occur in mammalian muscle (Takane, 1927). In no other tissue except for liver has the synthesis of glycogen from lactic acid been demonstrated. The conversion of lactic acid to glycogen in liver would appear to be much more firmly established than in muscle. Holmes & Ashford (1930) and Ashford & Holmes (1931) showed that lactic acid although disappearing cannot give rise to glycogen in brain. It would appear that this synthesis is not a general property of animal cells.

It has been said that more lactic acid disappears than is oxidized. The supposed ubiquity of this disappearance which is not accounted for was claimed to show the general application of Meyerhof's theory. The lactic acid oxidized is calculated from the difference between the oxygen uptake with and without lactic acid. This difference is supposed to represent the oxygen absorbed in the actual combustion of lactic acid. Ashford & Holmes (1931) suggested that the addition of lactate causes "sparing" or suppression of the oxidation of other substrates. If this were the case, one would not be justified in assuming that the difference between the oxygen uptake with and without lactic acid gives the amount of oxygen consumed in the combustion of lactic acid. If suppression of oxidation of other substrates does occur, the total oxygen uptake and not the difference would represent the oxygen absorbed in combustion of lactic acid. Thus more lactic acid than that calculated by Meyerhof and other workers would in reality be oxidized. This would reduce the oxidation quotient. Holmes & Ashford (1930) and Ashford & Holmes (1931) obtained data, using chopped brain, similar to those of Meyerhof on muscle and concluded that more lactic acid disappears from this tissue than can be accounted for by complete oxidation, although no glycogen synthesis could be demonstrated. They point out that the above criticism that "sparing" takes place does not affect their results, since in *some* cases more lactic acid disappears than is oxidized when the total oxygen uptake is used as a measure of lactic acid oxidation. It is not completely certain,

however, that pyruvic acid did not accumulate in some of their experiments. Quastel & Wheatley (1932*a*) showed that lactate spares the oxidation of succinate in brain and further that the addition of small amounts of lactate to brain respiring without substrate gives an excess oxygen uptake which would finally account for the complete oxidation of the lactate. This only proves (as indeed they admit) that complete oxidation may occur finally. Resynthesis in the Meyerhof sense may well have occurred at first, the resynthesized carbohydrate being later oxidized.

It is, however, improbable (see below) that the Pasteur effect really does occur in chopped brain, so that the question as to whether more lactic acid disappears than is oxidized under these conditions is actually irrelevant. In slices of cerebral cortex, on the other hand, the Pasteur definitely does occur (Dixon, 1936*b*), so that the fate of lactic acid in this case is a matter of importance to the present discussion. This question was investigated by Dixon (1935), who measured the disappearance of lactate from thin slices of brain cortex. It was first necessary to establish if addition of lactate suppresses the oxidation of other substrates. *Prima facie* this seemed likely since the oxygen uptake without substrate, although high at first, was found to approach zero in about 5 hours, while that in the presence of added lactate remains constant. If, on the other hand, sparing does not take place then the difference between the oxygen uptake with and without lactic acid would give the oxygen absorbed in oxidizing the lactic acid. This difference was found by Dixon to increase with time. Hence the lactic acid oxidized should increase with time. Accordingly there should be an increase in the rate of lactic acid disappearance as time progresses. However, the actual rate of disappearance of lactate does not increase with time. It remains remarkably constant. It was thus concluded that lactate addition suppresses the autorepiration and that the lactic acid oxidized should be calculated from the total respiration and not from the difference between this and the autorepiration. When this method of calculation is adopted, it is found that the lactic acid disappearing equals the lactic acid oxidized—in fact the lactic acid disappearing is equivalent to one-third of the oxygen uptake as required by the equation $C_3H_6O_3 + 3O_2 = 3CO_2 + 3H_2O$. It is thus clear that no more lactic acid disappears from brain cortex than can be accounted for by complete oxidation. The lactic acid which disappears is oxidized completely.

Another test was applied by Dixon (1935) to the theory that the Pasteur effect in brain cortex is caused by the rebuilding of lactic acid to carbohydrate in the presence of oxygen. Oxygen reduces the rate of lactic acid formation in brain from a $Q_M^{N_2}$ of about 20 to a $Q_M^{O_2}$ of between 0 and 2. If the Pasteur effect were caused by the rebuilding of carbohydrate from the lactic acid, the actual formation of which is masked by the presence of oxygen, then lactic acid added to brain should disappear at this high rate equal to the difference between $Q_M^{N_2}$ and $Q_M^{O_2}$. In fact the rate of disappearance of lactic acid should give a value of $Q_M^{O_2} = -18$. Instead of this values of -2 were found for $Q_M^{O_2}$. Therefore lactic acid cannot be formed with as high a velocity in oxygen as in nitrogen and then removed. The action of oxygen must be to reduce the gross rate of lactic acid formation and carbohydrate destruction, and must take effect somewhere prior to lactic acid formation.

Similar results¹ have been obtained with chick embryo. Here also the difference between the rates of production of lactic acid in oxygen and in nitrogen is much greater than the rate of lactic acid disappearance. It is clear, then, that in chick embryo as in brain cortex Meyerhof's theory of the mechanism of the Pasteur effect is not the correct explanation.

The demonstration by Long & Lupton and by Hill *et al.* (see above) that more lactic acid disappears in the whole animal after exercise than can be accounted for by oxidation, is open to the same criticism as is the work on muscle and brain. It is not at all certain that the excess oxygen uptake really gives a measure of the lactic acid oxidized. (It is however certain that lactic acid formed in exercise in the muscles can be resynthesized to glycogen in the liver (the Cori cycle). This would give an explanation for the Pasteur effect in whole animal but not in isolated tissues.)

To emphasize the lack of connexion between lactic acid disappearance and the Pasteur effect, we may mention the facts that the rate of disappearance of lactic acid is increased in brain cortex in the presence of $M/10$ potassium chloride (Dixon & Holmes, 1935) where the Pasteur effect is completely inhibited (Dixon, 1936*b*), while no lactic acid disappears when added to nerve (Holmes & Gerard, 1929; Holmes *et al.* 1930) in which tissue the Pasteur effect would appear to be in operation.

The evidence for Meyerhof's theory based on measurement of the Meyerhof quotient is of an extremely dubious nature, since in calculating the oxidation quotient from the Meyerhof quotient Meyerhof's theory is tacitly assumed. The fallacy in this assumption is patent from the following example. The Meyerhof quotient in brain cortex is usually about 2, the oxidation quotient should thus be equal to 6. We would thus expect six times as much lactic acid to disappear from brain cortex as is oxidized. In actual fact no more lactic acid disappears than is oxidized (Dixon, 1935).

We may thus conclude that the Meyerhof cycle occurs probably in amphibian muscle and certainly in the animal as a whole (Cori cycle), but that it certainly does not occur in mammalian brain cortex nor chick embryo and probably does not occur in isolated mammalian muscle and nerve, in all of which tissues the Pasteur effect operates. Further, even if the Meyerhof cycle is shown to occur in a tissue, it does not follow that Meyerhof's theory gives a correct explanation for the Pasteur effect. Meyerhof purports to have analysed normal resting respiratory metabolism into two stages, firstly, fission of carbohydrate to lactic acid as seen in anaerobiosis, and secondly, the partial oxidation of this lactic acid while the remainder is resynthesized. Thus the fact that the net loss of carbohydrate is less in oxygen than in anaerobiosis would be explained. This thesis can hardly be said to be proved in any single instance, since the fact that these two stages occur when a period of anaerobiosis or activity is followed by oxidative recovery does not necessitate that normal resting metabolism consists of these two stages. The Meyerhof cycle is thus a chain of phenomena which occurs to a limited extent in animal tissues. Where the cycle does not exist there must be some other explanation for the Pasteur effect and even in

¹ Unpublished observations of Dr J. Needham, Dr W. Nowinski and the author.

those tissues where this cycle does occur it is not certain that it is responsible for the Pasteur effect. This theory cannot therefore be a general explanation for the action of oxygen in reducing carbohydrate catabolism.

(2) WORTMANN'S THEORY

Wortmann (1880) considered that in plant respiration carbohydrates are first split to ethyl alcohol and carbon dioxide. The alcohol is then supposed to be oxidized and condensed to carbohydrate by a reaction represented by the equation $3\text{C}_2\text{H}_5\text{O} + 3\text{O}_2 = \text{C}_6\text{H}_{12}\text{O}_6 + 3\text{H}_2\text{O}$. This theory differs from that of Pfeffer (1878) in that Pfeffer supposed the alcohol to be oxidized completely to carbon dioxide and water. It is obvious that in the presence of oxygen the net loss of sugar is reduced below that in anaerobiosis owing to this oxidative resynthesis of sugar. Wortmann's theory thus gives an explanation for the Pasteur effect.

Wortmann supported his theory by the observation that the I/N ratio of certain seedlings is equal to unity. This substantiates the view that all the carbon dioxide produced in aerobic respiration is evolved in the preliminary fermentative stage and that the secondary respiratory stage, involving oxygen absorption only, causes resynthesis of carbohydrate, no carbon dioxide being evolved during the actual oxidation. If Pfeffer's view were correct that the alcohol is oxidized to carbon dioxide and water, the I/N ratio should be $\frac{1}{3}$.

Lundin (1923*a, b*) supported this view of Wortmann by showing that yeast assimilates more carbohydrate from sugar solution in the presence of oxygen than in its absence. He suggested that some of the alcohol is oxidized to reserve carbohydrate. Further Lundin found the carbon dioxide production in air and in anaerobiosis to be equal, the I/N ratio being 1. Lundin also found that less alcohol is formed in air than in anaerobiosis; this deficit is equivalent to the reserve carbohydrate synthesized. Later Lundin (1923*c*) showed that yeast can form carbohydrate when supplied with alcohol alone. This only occurs under aerobic conditions. Brücke (1933), however, has shown that the assimilation of glycogen from sugar is much faster than that from alcohol. He concludes that alcohol is not an intermediary in this assimilation from sugar.

That synthesis of carbohydrates can occur in plants from compounds formed by carbohydrate breakdown is evident from the work of Fürth & Lieben (1922*a, b*). They showed that lactic acid can form carbohydrate in yeast. Bennet-Clark (1932, 1933) has shown that more malic acid disappears from succulent plants than can be accounted for by oxidation. He assumes that synthesis of carbohydrate occurs. Later Bennet-Clark & Latouche (1935) showed that more citric and glycollic acids disappear from the medium surrounding the mould *Aspergillus niger* than can be accounted for by the amounts of these acids which are oxidized.

The main criticism of the Wortmann hypothesis is that in some cases even less carbon dioxide is produced in oxygen than in anaerobiosis. Here the I/N ratio is greater than unity. This follows from Meyerhof's work (1925) on yeast and was also shown for apples by Blackman & Parija (1928). In fact, as Meyerhof pointed out, the effect of oxygen in sparing carbohydrate is greater than could be explained by Wortmann's theory.

(3) MEYERHOF'S THEORY FOR PLANT METABOLISM

Meyerhof (1925) suggested that, instead of alcohol being resynthesized to carbohydrate by oxygen, the resynthesis takes place from some compound containing 3 carbon atoms and occurs prior to the formation of carbon dioxide. A certain amount of this 3-carbon compound is resynthesized to carbohydrate at the expense of energy derived from the oxidation of the rest. This theory would fit the facts better than that of Wortmann. Blackman (1928) also advanced a theory of mechanism involving resynthesis. Blackman's scheme does not specify whether a compound containing 2 or 3 carbon atoms is the substrate for resynthesis, although the results of Blackman & Parija (quoted above) would apparently agree better with Meyerhof's view than with that of Wortmann. Otherwise Blackman's theory is similar to those here described.

Meyerhof's theory for plant cells is substantiated better by actual demonstrations of carbohydrate resynthesis (see above under Wortmann's theory) than is this same theory as applied to animal cells. However there is no positive evidence that this resynthesis is actually responsible for the Pasteur effect.

We may conclude that it has not been definitely shown that the resynthesis of carbohydrate from the products of fission ever constitutes the real mechanism of the Pasteur effect. In brain cortex and embryo it is certain that removal of these cleavage products to form carbohydrate is not the factor responsible for the Pasteur effect. We will now consider the theories of mechanism which suggest that the gross rate of carbohydrate destruction is inhibited by the presence of oxygen.

(4) LIPMANN'S THEORY

It was suggested by Hahn *et al.* (1931) that the mere presence of oxygen inhibits the lactic acid production in muscle. Lipmann (1933*a*) supposed that oxygen reduces the gross rate of glycolysis by reversibly inactivating the glycolytic enzyme. He considered the enzyme partially to be oxidized to an inactive substance, so that the gross rate of carbohydrate catabolism is reduced in the presence of oxygen.

Although muscle extract and yeast juice, which contain myozymase and zymase respectively, are not inactivated by molecular oxygen, Lipmann (1933*a, b*) found that certain oxidation-reduction indicators in the presence of oxygen can cause this inactivation. Also oxidizing agents like iodine and quinone inhibit glycolysis. According to Frisch & Wilhelm (1934, 1935) the addition of reducing agents such as carotene and tumour "Kochsaft" increases glycolysis of muscle extract. They believe that even in the extract molecular oxygen causes inactivation. The increases recorded however are of a small order compared with the lactic acid originally present. Lipmann's work would suggest that atmospheric oxygen only reduces glycolysis in the presence of a suitable carrier. He maintains that in those cases where glycolysis is inhibited by oxidation-reduction indicators in the presence of oxygen, the oxidation-reduction potential of the whole system is raised. More negative indicators which have no effect on glycolysis do not raise the potential. Kluyver & Hoogerheide (1934) consider that in the living yeast cell the potential is similarly raised by oxygen

as in yeast juice in the presence of an appropriate carrier. They show that the potential is raised by oxygen in suspensions of those yeasts in which the Pasteur effect is marked. In pronounced fermenters on the other hand, in which there is little Pasteur effect, the potential is hardly raised by oxygen. Kluyver & Hoogerheide consider that the Pasteur effect is caused by an increase in the oxidation-reduction potential of the cell. The term "oxidation-reduction potential of the cell" is, however, meaningless,¹ since in the cell there is a whole range of potentials from that of the reducing substrates of the dehydrogenases up to that of molecular oxygen itself. The significance of the potentials observed by Kluyver & Hoogerheide is thus quite uncertain. Further it is just as likely that these potentials and the metabolic differences between the various yeasts are due to some common cause, as that the metabolic differences are caused by the differences in potential. Michaelis & Smythe (1935) maintain that even the action of dyes in inhibiting fermentation in yeast extracts is not dependent on the potential. Further in contrast to Lipmann they found this inactivation to be irreversible.

According to Lipmann's theory it is not the actual absorption of oxygen by the cell which causes the Pasteur effect, but the inactivation of the glycolytic enzyme by oxygen. The amount of enzyme inactivated would depend on the oxygen tension and not on the total respiration. According to Bumm *et al.* (1932) the oxygen tension does determine the Pasteur effect. This has been denied by Leiner (1935) (see Section III).

Geiger (1935) found that oxidized glutathione inhibits the glycolysis of both muscle extract and chopped brain. Further, the order of this decrease in the lactic production is the same as that produced by molecular oxygen. Geiger assumes the Pasteur effect to be working in chopped brain and suggests that it is caused by the oxidation of the glutathione present in the cells and the resulting inhibition of glycolysis. However, Geiger merely showed that oxygen reduces lactic acid production. He made no measurement of carbohydrate destruction. It is thus not certain from his results that the Pasteur effect is in operation. By the indirect method described in Section II one can calculate the rate of carbohydrate destruction in oxygen if one knows the respiration as well as the glycolysis. Geiger, however, made no measurements of respiration. If we calculate $Q_M^{N_2}$ and $Q_M^{O_2}$ from Geiger's figures, we find values of about 3 and 1.5 respectively for these quotients. The total anaerobic glycolysis and the decrease in glycolysis caused by oxygen in chopped brain are thus very low as compared with slices. We do know, however, by calculation from the results of Ashford & Holmes (1931) that the respiration of chopped brain is of the same order as that in slices. The net lactic acid production from carbohydrate is decreased in oxygen, but in oxygen the respiratory catabolism is superimposed. If this latter is taken into account it would appear that there is no decrease in carbohydrate catabolism caused by oxygen in chopped brain. The Pasteur effect is not working, and the decrease in glycolysis caused by oxygen is merely due to the removal of some of the lactic acid by complete oxidation. It is thus unwarrantable from Geiger's results to assume that oxidized glutathione is responsible for the Pasteur effect, for it is probable that oxygen cannot reduce

¹ As has been pointed out by Dr Malcolm Dixon.

carbohydrate catabolism under conditions where this can be effected by oxidized glutathione.

The view that the oxidative inactivation of some catalytic substance essential to glycolysis constitutes the mechanism of the Pasteur effect has been supported by some more recent work of Lipmann (1935) who found that xanthine oxidase inhibits fermentation by yeast juice and that the addition of boiled yeast or cozymase restores the original rate. Andersson (1935) also recorded the inhibition of fermentation and of muscle glycolysis by xanthine oxidase. Lipmann suggests that the oxidation of the coenzyme may be the mechanism of the Pasteur effect.

Lipmann's theory constitutes a real advance in our conception of the Pasteur effect in that it emphasizes the fact that the presence of oxygen reduces the gross rate of carbohydrate catabolism and does not act by causing resynthesis. It is, however, doubtful how far the experiments, which demonstrate that oxidizing agents can inhibit glycolysis in extracts, really substantiate the view that molecular oxygen has a similar action in the living cell. Further, it is exceedingly difficult to explain on Lipmann's theory how the numerous specific inhibitors of the Pasteur effect can inhibit the oxidation of a part of the glycolytic system without inhibiting respiration. Thus the addition of potassium ions, the removal of calcium and potassium ions, the addition of dinitro *o*-cresol etc. all inhibit the Pasteur effect in brain. On Lipmann's theory we would have to suppose that under all these different conditions the oxidative inactivation of some essential factor to glycolysis is specifically inhibited without any inhibition of respiration. It is difficult to conceive any such reaction being specifically affected by such diverse conditions.

(5) THEORY OF DIXON AND HOLMES

Dixon & Holmes (1935) suggested that the expenditure of energy by respiration is responsible for maintaining part of the cell enzymes inaccessible to the substrate. In anaerobiosis the cell becomes more permeable and the enzymes become more accessible to the substrate, thus the rate of carbohydrate catabolism is higher under these conditions than in the presence of oxygen. One can imagine the enzyme-containing part of the protoplasm as consisting of an emulsion in which the enzymes are in solution in the aqueous phase. The amount of the enzyme which is accessible to the substrate would depend on how much of the aqueous phase is continuous. When the aqueous phase is continuous throughout, then the total enzyme content is active. It is supposed that this is the case in anaerobiosis. In the presence of air part of the aqueous phase is discontinuous and hence part of the enzymes are inaccessible to the substrate. There is no doubt that anaerobiosis increases the permeability of brain (Spiegel and Spiegel-Adolf, 1936), of nerve (Cowan, 1934) and of muscle (Winterstein & Hirschberg, 1927). Further, that cell permeability depends on the phase conditions of an emulsion is a view which is widely held (see Clowes, 1916). It thus seems plausible to assume that the access of oxygen to the cell, which diminishes the permeability, will also diminish the amount of the emulsion in which the aqueous phase is continuous. In this way the amount of enzyme accessible to the substrate is diminished in the presence of oxygen and the Pasteur effect results.

According to the theory of Dixon & Holmes the enzymes which act on carbohydrates are not fully accessible in the normal oxygenated cell. Hence the metabolism of the cell is not at its maximum. We would thus expect respiratory catabolism as well as glycolysis to be limited in the normal cell. This view is substantiated by the fact that many inhibitors of the Pasteur effect (e.g. dinitro *o*-cresol and potassium chloride) cause an increase in respiration as well as raising the aerobic glycolysis. From this it follows that similar mechanisms are involved in suppressing lactic acid formation and in maintaining the normal and submaximal rate of respiration. If these mechanisms are injured carbohydrate metabolism runs riot in the cell. The restraining mechanisms are dependent, however, on energy supplied by normal respiration so that the catabolism of carbohydrates is limited in the normal cell in oxygen. The presence of potassium chloride in a concentration of $10^{-1}M$ removes both of these limitations in brain cortex. It is thus justifiable to assume that the same mechanism normally limits carbohydrate respiration as well as glycolysis. In brain cortex the addition of potassium chloride thus causes an increase in the respiration of glucose, mannose, fructose, lactate and pyruvate as well as the assumption of marked aerobic glycolysis with glucose and mannose. Lipmann suggested that the glycolytic enzyme is inactivated by oxygen. The work described above shows that in normal oxygenated brain cortex not only glycolysis but also the respiratory catabolism of carbohydrates is limited. This fact would make it probable that these reactions are all limited by some common physical factor such as the amount of enzyme containing protoplasm which is permeable to the substrate.

That this limitation of cellular metabolism is due to some physico-chemical factor is strongly suggested by the nature of the conditions which remove it. Thus excess of potassium ions remove the limitation in brain. This is also effected by rubidium and caesium though sodium and lithium have little effect (cf. the work of Finkle *et al.* (1923) on emulsions). Further the action of excess potassium can be antagonized by calcium. These ions antagonize one another in many physico-chemical systems (cf. the work of Bancroft (1913) and Clowes (1916) on emulsions). The removal of calcium from the environmental medium also causes similar changes. All these facts lead to the view that the rate of carbohydrate catabolism is dependent on some physico-chemical system in which ion antagonism plays an important controlling part. Further, the work of Stier & Stannard (see above) on the effect of grinding on the auto-fermentation of yeast leads to the idea that fermentation may be suppressed in oxygen by the physical architecture of the cell; when this is injured fermentation occurs.

Finally this theory can well explain inhibition of the Pasteur effect. In the first place, the addition of potassium chloride to brain directly makes accessible the total enzyme content of the cell. This may be effected by the inversion of an emulsion causing the whole of the enzyme-containing phase to become continuous and thus permeable to the substrate. Secondly, other inhibitors, which act in very small concentrations, may operate by inhibiting the respiration, which maintains the cell enzymes partly inaccessible and thus these inhibitors indirectly make the total enzyme content accessible. It has been suggested by Mendel (1930) that only part of

respiration is responsible for the Pasteur effect. Mendel found that dihydroxyacetone and acetol inhibit part of respiration without increasing glycolysis. Presumably the part of respiration not concerned with the Pasteur effect is inhibited. Dixon (1936*a*) similarly suggested that specific inhibitors of the Pasteur effect only reduce the part of respiration concerned with the Pasteur effect. Thus dinitro *o*-cresol is supposed to abolish this specific fraction of respiration. The whole of the cell enzymes become accessible and there is therefore an increase in glycolysis and in the remainder of respiration. There is thus a primary inhibition of the specific fraction of respiration which causes the Pasteur effect and a secondary augmentation in the remainder. The net effect on the total respiration is determined by the relative magnitudes of these changes. In the above case the primary inhibition is less than the secondary augmentation and there thus is a net increase in respiration. In other cases there may be a larger initial inhibition of respiration and the two effects may cancel out so that no net change in respiration is observed to accompany inhibition of the Pasteur effect, as with phenosafranine (Dickens, 1935). Again with higher concentrations of the inhibitor or with more toxic substances (e.g. cyanide) nearly the whole of respiration is initially inhibited. It is interesting to note that in low concentrations cyanide may cause no net change in respiration although inhibiting the Pasteur effect (Genevois, 1927*b*; Laser, 1935), or it may even cause an increase in respiration (Warburg, 1919). It is thus seen that the theory of Dixon & Holmes can explain the respiratory changes accompanying inhibition of the Pasteur effect.

(6) THEORIES INVOLVING THE OXIDATIVE REMOVAL OF SOME ESSENTIAL INTERMEDIARY IN THE GLYCOLYTIC CHAIN

It might be supposed that carbohydrate catabolism could be checked owing to the removal by oxygen of some essential intermediary in the chain of reactions by which lactic acid is formed. Thus it was suggested by Holmberg (1934) and by Ashford & Dixon (1935) that the removal of α -glycerophosphate by oxygen might break the glycolytic chain. This view is improbable since Ashford & Dixon found that neither the addition of α -glycerophosphate nor pyruvate can increase aerobic glycolysis. Similarly it might be supposed that the oxidative removal of acetaldehyde (a substance whose presence is necessary for the continuation of alcoholic fermentation) might be the cause of the Pasteur effect. Unpublished observations have shown, however, that the addition of acetaldehyde in concentrations which do not inhibit respiration do not increase the aerobic fermentation of baker's yeast. Boyland & Boyland (1935) suggested that the removal of triose phosphate might similarly check lactic acid production. This, however, would hardly explain the decrease in carbohydrate catabolism.

We thus see that there is no positive evidence for a mechanism of this type containing the true explanation for the Pasteur effect. Further, on this type of theory it would be almost impossible to explain inhibition of the Pasteur effect.

V. SUMMARY

1. The Pasteur effect is defined as the action of oxygen on living cells which reduces the rate of carbohydrate destruction and suppresses or diminishes the accumulation of the products of anaerobic metabolism.

2. This effect is best measured by direct estimation of the rate of carbohydrate destruction, but can be evaluated indirectly from simultaneous measurements of the respiration and the accumulation of fission products.

3. The Pasteur effect apparently depends on the actual absorption of oxygen by the cell. If respiration is eliminated the Pasteur effect is abolished. However, under certain conditions which are described, the Pasteur effect can also be abolished when respiration is not reduced in magnitude.

4. Two kinds of mechanism have been suggested for the Pasteur effect. Firstly, it has been assumed that in oxygen the gross rate of carbohydrate destruction is not diminished below that in anaerobiosis, but that some of the products of anaerobic fission are resynthesized to carbohydrate in the presence of oxygen. In this way the net rate of carbohydrate destruction is diminished. Secondly, it has been suggested that oxygen in various ways diminishes the gross rate of carbohydrate destruction. The first type of explanation has been shown to be inapplicable to the metabolism of certain tissues and we may conclude that in these at any rate the presence of oxygen reduces the gross rate of carbohydrate catabolism. This is probably the general explanation for the Pasteur effect. The limiting action of oxygen may either be due to its effect on the physical state of the cell causing inaccessibility of the enzymes, or else may result from oxidative inactivation of the glycolytic system.

The author is indebted to Mr Alan Hodgkin for valuable advice and criticism and for suggesting that potassium stimulates the metabolism of brain in aerobiosis and inhibits in anaerobiosis owing to a difference in permeability under these two conditions; he also wishes to thank Mr Kenneth Harrison for a number of suggestions.

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PROBLEMS OF HEREDITY IN THE LEPIDOPTERA

By E. B. FORD, M.A., B.Sc.

(Lecturer in Genetics, and University Demonstrator in the
Department of Comparative Anatomy, Oxford)

(Received 10 July 1936)

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I. INTRODUCTION

A GENERAL survey of the genetics of the Lepidoptera would occupy a larger space than can be allowed for this article, unless it were too condensed to be useful. I have therefore restricted it to a consideration of the effects of the individual genes so far studied in these insects. It will be realized, however, that they have also provided material for many important genetic investigations on other lines; especially those relating to species-crossing, fluctuation in numbers, the induction of mutation, phenotypic variation (in particular, by means of temperature experiments), and the analysis of sex-determination. Logically, the last of these subjects ought to be included in this discussion, for there is no real ground for separating factors controlling sex from any others and, indeed, their study has been particularly helpful in illuminating genetic physiology as a whole. But there are good reasons for omitting any consideration of them here. The celebrated work of Goldschmidt on intersexuality in *Lymantria dispar* L., has now been concluded and practically brought to completion, and he has himself summarized it in English in a final monograph which covers the whole subject in detail (Goldschmidt, 1933 c). Furthermore, though a number of highly interesting problems are also presented by the sexual abnormalities which have been encountered in other species of

Lepidoptera, it is understood that a survey of them will shortly appear in *Biological Reviews*. Consequently it would be superfluous to develop any of the aspects of sex-determination in the present account.

It will probably be more valuable to discuss some of the important questions raised by genic action in this order than to give a descriptive catalogue of the genes so far detected in it. For the Lepidoptera are in fact particularly suited to the elucidation of certain genetic problems of the highest interest, while they do not provide appropriate material for others which have so far received the greatest share of attention. Thus the large number and small size of their chromosomes do not favour a study of the physical basis of heredity: nor does the fact that hardly any of the species produce more than two or three generations in a year, while great numbers are but single-brooded. The holometabolous nature of these insects also places restrictions on certain lines of inquiry, notably those relating to the action of genes controlling the rate of development of the imaginal characters. On the other hand, many aspects of genetic physiology may be approached with exceptional facility in this group. Indeed it has been used extensively for this purpose by the German school of geneticists, especially by Goldschmidt and his colleagues, whose work has been of the highest value. Furthermore, probably no other animals provide such good opportunities for the study of genetic factors in nature. For the recent spread of melanism among many of the species in and around industrial areas constitutes an evolutionary change of an almost unparalleled kind. Moreover polymorphism is of rather frequent occurrence especially among the butterflies, but it is also well known among moths. It is maintained by genetic control of varying complexity, and helps to throw some light on the way in which selection can affect genotypic variability.

Although I do not claim that all the genetic factors which have been studied in the Lepidoptera are recorded here, only a small proportion of them is likely to be omitted. Indeed I have endeavoured to incorporate as many of them as possible into this account, logically and without repetition. Consequently it has seemed worth while to provide it with an index, from which students can readily find what factors are known in any species.

An important exception has, however, to be made.¹ More genes have been studied in the silkworm (*Bombyx mori* L.) than in any other species of the order. But these present rather a special problem. The moth is unknown in a wild state, and much of the experimental breeding which has been carried out on it relates to the commercial production of silk. In these circumstances, the disproportionately large space which it would occupy here seems hardly justified. An up-to-date account of its genetics is much better suited to treatment in a separate monograph, such as those published from time to time in *Bibliographia Genetica*, and elsewhere. I have therefore decided to omit the species from this paper. A second, and personal, consideration has also influenced me in this matter. A great deal of work on the silkworm has been done in Japan, and much of it has been published in Japanese. Consequently a knowledge of that language seems a necessary qualifica-

¹ See also footnote on p. 478.

tion for anyone who attempts to summarize silkworm genetics, and it is one which I do not possess.

I am most grateful to Mr N. D. Riley, Keeper of Entomology at the British Museum (Natural History), to Mr W. H. T. Tams, and to Mr L. B. Prout for their kindness in checking and bringing up-to-date the generic and specific names used in this article. These are, therefore, in accord with the most recent advances in nomenclature. Many of the names still widely used in the literature are in reality obsolete, and those employed here must be substituted for them. For the benefit of workers who have not been able to keep in touch with such changes, discarded names are given in addition if they have become very well known. They are then placed in brackets at the first mention of the species in question, and they are also included in the index. The author of each name is given the first time it is used.

Mr Riley informs me that no modern and authoritative basis at present exists for the nomenclature of varieties and forms subsequent to Seitz, *The Macrolepidoptera of the World*, which I have accordingly used for this purpose. Those which are more recent than the appropriate part of that work are from original descriptions.

I am greatly indebted to Prof. J. S. Huxley for his advice, and for making a number of valuable suggestions. My grateful thanks are due to Prof. E. S. Goodrich and Sir Edward Poulton for their kind help, and to Prof. G. D. Hale Carpenter for his help, and for placing at my disposal the resources of the Department of Entomology at Oxford. I also wish to express my thanks to Dr R. Hanitsch for the valuable assistance which he has given me.

II. HEREDITY IN THE ABSENCE OF VISIBLE SEGREGATION

The inheritance of a character is most easily studied by crossing two individuals in which it is sharply contrasted. But it sometimes happens in the Lepidoptera, as in other forms, that this leads to no distinct segregation in any subsequent generation. On the contrary, the offspring may prove to be intermediates, while variation in the F_2 generation may form a "continuous" series from one grandparental type to the other, or an approach thereto.

Thus Prout & Bacot (1909) crossed the typical grey London form of *Ptychopoda seriata* Schr. (*Acidalia virgularia* Hb.) with the whitish race (*canteneraria* Bdv.) from the south of France, and bred as many as ten generations. They, however, obtained only a gradation of forms, without any clear-cut segregation. Geographical races will often differ from one another in a number of genetic factors, leading to precisely this result: a conclusion not clearly appreciated by these authors. It is noteworthy that one of the pairs of allelomorphs was susceptible of individual study in the present instance. Alexander (1912) showed that the dark speckling, which is among the characteristics of the English race, behaves as a single dominant. Its effect in the heterozygote was, however, somewhat irregular: not an unexpected result when considerable recombination is taking place in the gene-complex.

The cross between normal and melanic *Oporinia dilutata* Bork. leads to the production of an unclassifiable series from one extreme to the other in subsequent

generations (Harrison, 1920a). Hawkes (1922) worked on cocoon colour in the saturniid moth *Philosamia cynthia* Drury, crossing two races: *cynthia* and *ricini* Bsd.¹ The former spins reddish brown, and the latter pure white, silk. Various intermediate shades appeared in F_1 and F_2 , passing imperceptibly from the light to the darker form. Goldschmidt (1932, 1933c) has studied the length of the diapause in *Lymantria dispar* L.; in this species the duration of the period passed as an egg. This is a highly adaptive character, timing the hatching of the larva with the appearance of the young leaves. It is not wholly controlled by temperature, being in part genotypic; but a cross between races having long and short periods respectively leads to no definite segregation.

Such results as these have given ground for some misconception as to the kind of heredity involved, being generally regarded by the earlier workers as "non-Mendelian". Now all theories of bisexual inheritance can be divided into two main types, depending on whether they rely upon a blending or a particulate mechanism. That is to say, the factors controlling the characters may either themselves blend, or remain permanently distinct. The former is true "blending inheritance". In reality it underlies most of the hereditary devices ever postulated: the arguments of Darwin assume inheritance of this kind. Very different is the particulate theory introduced by Mendel; for it preserves the genotypic variability of the organism, which, on the contrary, a blending mechanism must halve at every generation. Thus the two fundamental types of bisexual inheritance may always be distinguished experimentally by their very different effects on variability. If the F_2 generation from any cross is more variable than the F_1 , then the heredity involved is not blending but particulate, that is Mendelian, whether it leads to any visible segregation or not; a proposition which seems first to have been stated in this form by Fisher (1930).

As pointed out by Fisher (1930), it is extremely improbable that any significant part of organic inheritance depends upon factors which blend. Indeed the greater variability of the second hybrid generation would no doubt be detectable in all the instances so far mentioned, and in some of them it was sufficiently marked to attract attention at the time. Thus it was recorded by Hawkes; also by Goldschmidt, who definitely regards the length of the diapause as controlled partly by multiple factors, which he conjectures to be but few in number.

The term "continuous variation" is not unexceptionable. It may, however, be usefully defined as variation at least no greater than that which, on the average, separates parent from offspring. If the multiple factors controlling it exhibit dominance, and this is exerted chiefly in one direction, it will depart from the approximately normal distribution which it would otherwise attain and fall within a skew curve. An instance of this kind is provided by the work of Hawkes (1922), who studied imaginal characters, in addition to cocoon colour, in his saturniid cross (*Philosamia cynthia cynthia* \times *P. cynthia ricini*). He found that the majority of the scales were like those of *P. cynthia cynthia* in structure both in F_1 and F_2 and, further, that they were subject to much greater variability in the latter generation.

¹ Hawkes treats these as distinct species, but Seitz (1908b) regards the latter as a small domesticated race of the former.

The labours of Goldschmidt have brought to light several examples of maternal inheritance in *Lymantria dispar*. Thus the length of the diapause sometimes shows a matroclinous effect (Goldschmidt, 1933*c*), although it is also influenced by multiple factors segregating normally. Further, the larval coloration seems to depend wholly on the mother in the early instars (Goldschmidt, 1924*a*), after which it comes under the control of a single pair of allelomorphs (p. 469). These results may be due to factors situated in the Y-chromosome, which is, of course, restricted to the female in the Lepidoptera and is known to be present in this species. However, Goldschmidt seems to have succeeded in ruling out this possibility in one instance. For he has shown that the female-determinant (*F*), detected in his work on intersexuality, is carried in the cytoplasm; consequently the offspring receive it only from their mother. The details of the very ingenious test by which he arrived at this conclusion are described in his final monograph on *Lymantria* (Goldschmidt, 1933*c*), to which reference has already been made. They are therefore outside the scope of this article. I have elsewhere given reasons for thinking that true cytoplasmic heredity, such as this, is unlikely to contribute any considerable share to organic inheritance as a whole (Ford, 1934).

It may be added that Harrison and Main (1908) have demonstrated maternal inheritance in a cross between *Pieris napi* L. and *P. bryoniae* O. In the former the ground-colour of the females is white, and in the latter yellowish with a heavy suffusion of black. The hybrid females on the whole resemble their female parent whichever way the cross is made. However, Müller (1933) has provided considerable evidence to show that *bryoniae* is a distinct species, instead of an alpine and arctic form of *napi*. Consequently it is possible that abnormalities of segregation may result from the cross between them, so that conclusions on the type of inheritance involved ought not to be reached without cytological investigation.

III. THE GENETICS OF LARVAL CHARACTERS

The exceedingly diverse larvae of the Lepidoptera provide excellent material for the study of variation in this phase. Here we shall be concerned entirely with the genotypic side of the problem. But a most valuable survey of its other aspects may be obtained from an article by Cockayne (1927-8). This contains numerous observations of great interest, many of which would well repay further investigation.

It is remarkable that the action of the genes responsible for larval variation is usually restricted to this stage only in the life history. Even when similar colour varieties are found in the larva and imago of the same species, they are generally controlled quite independently: a fact which will become clear from the following survey.

Poulton (1927) discovered an albinistic variety of the larva of *Abraxas grossulariata* L. All the black markings, except those on the head, were absent, but the usual yellow stripe was retained. Such individuals were very weakly and generally died, but the results indicated that the condition was uni-factorial and recessive. The larvae of the Sphingidae usually possess a long posterior dorsal horn, but occasional

specimens have been found in which it is reduplicated. Cockayne & Hawkins (1933) have studied this condition in *Sphinx ligustri* L. and shown that it is recessive, and controlled by a single factor pair. They obtained an F_2 generation of 60 normal and 18 two-horned larvae.¹ We may feel confident that the presence of two horns in the larvae of *Amorpha populi* L. has a similar genetic basis; for Howard (1930) captured a pair of this moth in London which produced about 80 larvae, of which about 25 had two horns. A gene reducing the size of the tubercles appeared in F_2 from the cross *Samia cynthia cynthia* \times *S. cynthia ricini* made by Hawkes (1918). The character is recessive. Cockayne (1931) has given good reasons for concluding that an abnormality in the larval pattern of *Mamestra pisi* L. is genotypic, though its precise nature is not known. He has also shown (1932*b*) that the absence of the yellow spiracular stripe in the larva of *Pheosia dictaeoides* Esp. appears to be a simple dominant. In none of the foregoing instances have the moths resulting from the abnormal larvae shown any peculiarities.

Some of the important problems raised by the occurrence and spread of melanism in imaginal Lepidoptera will be discussed in the section devoted to that subject. But it must here be noticed that melanic larvae also occur. In recent years they have appeared in species in which they were previously unknown, and have become common in some districts where formerly the normal form only could be found. Harrison (1932*b*) gives an excellent account of the spread of larval melanism in northern England, stating the species in which it has been detected, the date of its first appearance in each, and its present status. In the majority of instances nothing is known of its genetics, and there is an interesting field for inquiry here. However, he has shown that it is a single recessive in *Selenia bilunaria* Esp., and that it appears to be a simple dominant both in *Meganephria oxyacanthae* L. and *Abraxas grossulariata*,² though this is not yet quite certain. The moths reared from these larvae are normal, though melanism is known in the imago of all three species. Similarly, though the melanism of *Lymantria monacha* L. has a multi-factorial basis in the adult moth (pp. 481-2), that of the larva is quite independent of it and due to a single autosomal dominant (Goldschmidt, 1921). The Eurasian larvae of *L. dispar* are dark, but this is a racial character not really comparable with the melanism here discussed, of which there is no clear instance in the larva of this species. However, the presence on it of a black dorsal stripe is due to an autosomal dominant (Klaas, 1919), and unconnected with the melanism of the moth (p. 482).

Very few instances are known to me in which a gene has been reported to affect both larval and imaginal characters. One of these occurs in *Lasiocampa quercus* L.; a moth with a reddish brown body, and wings of the same shade crossed by a yellowish band. In var. *olivaceo-fasciata* Cockll. the reddish tint is lost throughout, so that the wings and body are darkened and the bands assume an olive hue. Cockayne (1927-8) states that it is inherited as a single recessive, and that the colour of the larval fur, which normally has the same reddish tint as the

¹ These figures are transposed in their table of results, but Dr Cockayne has kindly informed me that this is due to a misprint.

² In this species dominance may be incomplete. Intermediate larvae occur which are probably heterozygotes, but they are nearer the melanic than the normal form.

moth, is similarly affected in this variety. However, Hughes (1931-2) found that not all his dark larvae produced olive-coloured moths, and that one normal larva did so. It appears that some relation exists between the two conditions, as they are at least generally associated. The effects of the gene may be variable, or there may be linkage between two distinct genes having analogous effects in the different instars.

Two other, and definite, examples in which a gene affects both larva and imago have both been elicited by the work of J. H. Gerould on the American *Colias philodice* Godt. Typically, the larva, pupa, and eye of the imago of this butterfly are of a grass-green shade. Gerould (1921), however, encountered an autosomal mutation in which they are blue-green in colour. Normally, yellow xanthophyll and blue-green chlorophyll- α are both present in the blood, being extracted from the food. Physiologically, this gene acts as an inhibitor of the former, preventing its digestion, but not of the latter. This affects the blood in all stages and, consequently, the tint of those structures coloured by it: even the eggs are white instead of yellowish, owing to the lack of xanthophyll in the maternal blood. The larva of the blue-green variety is as healthy and as resistant to disease as the normal form; but the imago is less active, does not pair so readily, and is less fertile. Its normal allelomorph has an interesting adaptive significance, for by making the larva and pupa grass-green, it matches them accurately to the colour of their food plant. Gerould showed that this effect has real selective value, for the normal form is protected against the attacks of birds, while the recessive is not. The blue-green larvae were eliminated by English sparrows from a mixed stock kept out-of-doors.

Gerould (1926) has also studied the effects of a second and somewhat similar mutation in this butterfly. It has a reddening effect on the xanthophyll, so converting the yellow-green pigments to an olive green shade. This is rather less striking in the pupa, and somewhat more so in the eye of the imago, than is the change to blue-green. Unlike the latter, neither capacity to mate nor fertility is reduced in the present form. Both varieties are uni-factorial and recessive, and the genes controlling them are inherited independently. But since the olive green is due to a modification of xanthophyll, it cannot be detected in the blue-green type, in which this pigment is absent. The dihybrid cross therefore gives a ratio of 9 : 3 : 4 in the F_2 generation. But the gene producing the olive green coloration has a second and apparently distinct effect. For it influences the colour of the scales on the underside of the hindwings and the tips of the forewings, giving them an orange hue in the males and the yellow females, and a buff shade in the white females (p. 490). This change is equally marked in the homozygous blue-greens, so that, by its use in addition, the segregation of the dihybrid F_2 into a ratio of 9 : 3 : 3 : 1 can be detected.

In two of the instances just cited, the similarity in variation at different stages in the life history can be traced to the genetic control of a single substance. In the meal-moth, *Ephestia kühniella* Zell., the normal form has black eyes and testes, and a reddish larva. The condition with red eyes, testes not black, and a white larva, is recessive (aa). It has a low viability. However, a recessive gene producing trans-

parent eyes (*tt*), without effect except in the presence of *aa*, restores the viability of the latter to normal. A multiple allelomorph (*a^k*), producing brown eyes, is also known at the *a* locus (Kuhn & Henke, 1929-36).¹

We need feel no surprise that the characters of the larva and the imago are generally determined independently. On evolutionary grounds, any necessary association between them would usually be harmful; for even variation on similar lines would generally have a very different adaptive value in these instars. On the genetic side, it has only been possible to obtain very vague indications of the total number of genes available in higher organisms. But, on the whole, these suggest that the figure is large. Thus "Student" (1933) analysed the data obtained by Winter (1929) on selection for protein content in maize, and reached a lower value of 100-300 as the number of genes responsible for this one character; and he adds that some thousands are probably concerned.

It seems, then, that the independent control of larval and imaginal characters presents no exceptional problems. The genes responsible for both must, however, all be regarded as forming a part of the total gene complex, resulting in an interacting system influencing as a whole the effects of each of its components. The type of genotypic variability so produced has in recent years been brought into prominence, notably through the theory of dominance-modification elaborated by Fisher (1931).² This is a contribution of the highest importance to genetic theory. It postulates that dominance and recessiveness may be arrived at by selection operating on the gene complex: the effects of a given gene becoming recessive if disadvantageous and dominant if favourable, while the gene itself remains unaltered. Consequently, though the genes are subject to sudden mutation, the characters for which they are responsible may undergo slow selective modification. Such changes as these can only be brought about if a species has considerable experience of the gene in question. Therefore, as Fisher has pointed out, one of the most important tests of this theory is supplied by the reaction of genes when placed in internal environments to which they are unaccustomed. For dominance should then fail if it has been reached by the adjustment of the species to the gene; not so, if it were a property of the gene itself. The Lepidoptera provide excellent material for tests of this kind, and at least one relevant instance may be cited from the work so far carried out on larval characters in this group.

The larva of *Philosamia cynthia cynthia* is spotted, and that of *P. cynthia ricini* is not. Hawkes (1918) has shown that the former condition is incompletely dominant in the *F*₁ hybrid larvae, which range from partly to fully spotted forms. But such an observation as this is of little direct value in the study of dominance, since we know nothing of the normal genetic control of spotting in the larva of either species. This criticism is, however, met in some very similar observations of Federley (1911) on species-crossing in the genus *Pygaera*. The larva of *P. anachoreta* Fb. normally possesses a white spot on the first abdominal segment. A

¹ These comprise the only factors known to me which affect the eye colour in Lepidoptera, with the exception of an autosomal recessive producing white eyes in *Lymantria dispar* (Lenz, 1922).

² *Biological Reviews*.

non-spotted variety of it has been found, and this condition behaves as a simple and complete recessive. The larva of the allied *P. curtula* L., on the other hand, always lacks this spot. Although present, it is not fully developed in the hybrid larvae resulting from a cross between the latter species and (spotted) *P. anachoreta*. That is to say, the dominance of the gene for spotting becomes incomplete when immersed in a gene complex half of which is derived from a species unaccustomed to it. In a review of these results, Sturtevant (1912) discusses this larval spotting and remarks: "Its behaviour in this hybrid is somewhat complicated, and more data will probably be required in order to explain it. But Federley's assumption of imperfect dominance in the same gene which behaves as a complete dominant in the *anachoreta* mutant hardly seems justifiable." Facts which appeared contradictory in 1912 have become perfectly consistent in the light of Fisher's subsequent work.

It is instructive to compare the above result with that obtained by introducing into a hybrid a dominant gene having similar effects in both parental species, and probably identical in them. The dominance is then not impaired (p. 490). The facts discussed on pp. 484-5 should also be considered in relation to this problem.

The geographical races of a species often differ from one another in their larval characters, but so far these have been analysed in but few instances. The larva of *Lasiocampa quercus* has red fur in Sicily (var. *sicula* Stgr.) and white fur on the Riviera (var. *meridionalis* Tutt). This difference is uni-factorial, red fur being dominant (Bacot, 1901). In *Lymantria dispar* the Eurasian larvae are dark, and those from south-western Japan are light. Goldschmidt (1924a) has shown that the latter condition is incompletely dominant in the young, but the older heterozygotes become as dark as the homozygous dark parents. It has already been mentioned that the F_1 larvae are sometimes matroclinous at first. In F_2 a monohybrid 1 : 2 : 1 ratio is obtained. This is the first instance in which Goldschmidt was led to postulate the existence of genetic factors controlling the rate of a process in the body. The dark larvae may be regarded as having a pigmentation gene which operates early, so that all the young are dark, while its allelomorph in the light races acts so slowly that a sufficient amount of pigment is not formed during development. The process proceeds with intermediate speed in the heterozygote. It does so too in the race from northern Japan, where the larvae are at first light and darken later. This is due to a multiple allelomorph.

The races of *L. dispar* also differ in the number of moults which the larvae undergo. Goldschmidt (1933a) considers that moulting is controlled by a series of three multiple allelomorphs T_1 , T_2 , and T_3 . The first of these (T_1) produces four moults in both sexes; T_2 , four in the male and five in the female; while T_3 induces five moults in both sexes. The dominance of T_1 is incomplete. The larvae in some heterozygous broods ($T_1 T_3$) moult four times only, in others a few may moult five times. Goldschmidt holds that T_1 induces fast initial growth in the female, making it more male-like, while T_3 inhibits initial growth in the male, making it more female-like. The T_1 and T_2 allelomorphs both occur in Europe, but T_3 is absent there. In the northernmost island of Japan, Hokkaido, only T_2 is found, and in the Tokyo region only T_3 .

The larval characters of Lepidoptera have been studied much less thoroughly from the genetic standpoint than have those of imagines. However, it will be apparent from this brief survey that they present features of considerable interest, which would well repay investigation in the future. Thus, although so generally under independent major control, the genes operative in the one stage doubtless affect in some degree the action of those mainly responsible for the expression of characters in the other. In this connexion the relation of larval to imaginal melanism deserves careful analysis. Furthermore, larvae provide an opportunity for the study of rate factors which is wholly lacking in the perfect insects. It would, indeed, be very instructive to examine the effect upon various characters of genes speeding up or slowing down larval development.

IV. LINKAGE

The Lepidoptera is one of the few groups in which the female is the heterogametic sex. This was discovered on genetic grounds, but it has since been confirmed cytologically by Kawaguchi (1933). The chromosome numbers are rather large, ranging from a diploid value of 26 to 62 in those species so far studied (Schrader, 1928; Seiler & Haniel, 1921). This is perhaps the reason why so few sex-linked factors have as yet been detected in these insects. I am acquainted only with the following instances of them.

As is well known, the pale variety *dohrnii* Koenig (*lacticolor* Raynor) of *Abraxas grossulariata* was proved to be a sex-linked recessive by Doncaster & Raynor (1906), being the first demonstration of this type of inheritance. Sir Edward Poulton has bred a distinct strain of this form, in which the reduction of the markings is less extreme above, while they are not suppressed below, and the ground colour is without any creamy tint. It also behaved as a sex-linked recessive, and may have been due to another allelomorph at the *dohrnii* locus. Unfortunately it was never crossed with typical *dohrnii* and, as it has become extinct, the point cannot be settled. It may be mentioned that a form which seems phenotypically identical with Poulton's was investigated by Woodlock (1916), who called it the "Q" variety. Rather curiously, however, it is genetically distinct, being an autosomal recessive. Goldschmidt (1921) has studied a sex-linked dominant in *Lymantria monacha* which produces a great extension of dark pigment on the forewings, and uniformly blackish hindwings. It combines with two autosomal genes to affect the more extreme degrees of melanism in this species (pp. 481-2). Harrison (1920a) has shown that the dark-banded form *latifasciata* Vrbdt. of *Oporinia autumnata* Bkh. is sex-linked, with dominance incomplete. The (male) heterozygotes are intermediate, but nearer the normal form. The presence of a dark patch on the forewings of *Ephestia kühniella* is inherited as a sex-linked recessive (Kuhn & Henke, 1929-36). Furthermore Federley (1911) has produced some evidence that the factors controlling larval coloration in the genus *Pygaera* are, at least in part, sex-linked. It seems possible that *P. curtula* and *P. anachoreta* possess different allelomorphs of them. The larva of the former is light and of the latter dark. In the cross *curtula* ♂ ×

anachoreta♀, the F_1 hybrid larvae are more like *anachoreta* in the male and *curtula* in the female. Only males were obtained from the reciprocal cross, and these resembled their male *anachoreta* parent.

An interesting possibility is raised by the work of Mr H. B. Williams on *Boarmia rhomboidaria* Schiff. (= *gemmaria* Bkh.). His investigations are largely unpublished, but he has generously placed his records at my disposal and allowed me to quote from them. On a number of occasions he has obtained the yellowish form, *haggarti* Williams, segregating in his families, and his results indicate that the effects of the *haggarti* gene cannot be expressed in the black var. *rebeli* Aigner, which was also being used in his crosses.

Mr Williams's stock of *haggarti* originated from a female which he captured in Ayrshire. From this individual he bred 5 specimens, all males. One of these was mated with a female *rebeli*, and 46 specimens of *haggarti* have segregated at different times in the succeeding generations, 44 of them being females. The analysis is complicated by the presence of *rebeli* but, apart from the 5 males mentioned above, the results are consistent with the view that *haggarti* is a sex-linked recessive. The 2 other *haggarti* males appeared, with 7 females of this form and 3 normal males, as the offspring of a *haggarti* female and a normal male. The latter was paternally descended from *haggarti*, and was therefore probably a heterozygote. Unfortunately this is the only occasion on which *haggarti* has been bred from parents neither of which was *rebeli*.

The most plausible assumption is that we here have to deal with occasional crossing-over between the X- and Y-chromosomes. When this phenomenon is rare, it will lead to results of the kind encountered in this work. When common, it will cause sex-linked genes to approach the behaviour of autosomals. The latter situation is discussed by Darlington *et al.* (1934), who appeal to those engaged in the genetics of mammals to provide data on sex, in the hope of detecting it. It is very desirable that entomologists should also keep a watch for this type of inheritance.

Autosomal linkage seems to have been demonstrated with certainty only once in the Lepidoptera. Fryer (1928) has shown that the presence of a black costal blotch in *Acalla comariana* Zell. is due to a dominant autosomal factor (C), whose recessive allelomorph (c) produces a brown blotch. There is close linkage between this gene and the series of multiple allelomorphs (B) controlling the ground colour (p. 482). That no more examples of this kind have been found is no doubt due to the infrequency with which dihybrid ratios have been studied in this group. There is, however, some suggestion that linkage may exist between the genes controlling the forms *hospita* Schiff. and *subalpina* Schaw. of *Parasemia plantaginis* L. (see p. 492).

V. GEOGRAPHICAL VARIATION

Geographical races should be regarded as forms wholly characteristic of a particular region. As such, they have seldom been studied from a genetic standpoint in the Lepidoptera. Quite often, however, a given variation is found com-

monly but not exclusively in one or more districts, and is rare or absent elsewhere. Such instances are more appropriately considered in relation to polymorphism; while melanism, which has affected the whole population of some species in certain areas, must be treated as a separate problem.

The moth *Diaphora mendica* Clerck is sexually dimorphic throughout the greater part of its range. The males are sooty brown and the females white, but both have small black dots scattered over the wings. In var. *rustica* Hb., however, the ground colour of the males is whitish or yellowish in tint. It occurs to the exclusion of the normal *mendica* in Ireland, and in a few districts on the Continent. The females of the two forms are not separable.

It is now known that the difference between these races is due to a single factor pair, without dominance. The heterozygous males, called var. *standfussi*, are subject to a wide range of variation, but they are usually intermediates of a sandy colour. Occasionally, however, they become so light as to overlap *rustica* or so dark as to approach, but not to overlap, *mendica*. These facts have been elicited by the work of Adkin (1927) and Onslow (1921*b*), who have crossed the two forms in both directions, and raised large numbers of the F_1 , F_2 , and both types of R_2 generations. Cockayne (1919) had concluded that the difference between them is not unifactorial, but he had bred very few specimens (only 5 males in F_2), and other data then available were few. There is now no reason to doubt the simple interpretation adopted here. It would, however, be interesting to carry out a selection experiment on var. *standfussi*. Very probably its variability would prove to be genotypic, as is that of the variable heterozygote between *Diacrisia lubricipeda* Esp. and its variety *xatima* Cr. (pp. 479-80), a condition illustrating how dominance may be attained.

We may now consider the work carried out on geographical variation in the imago of *Lymantria dispar*. Although the various Japanese races of this moth differ greatly in intensity of wing colour, those from Europe can always be separated from them owing to their characteristic greyish tint. This is due to a sprinkling of white scales, which Goldschmidt (1933*c*) attributes to a single factor (G), not allelomorphic to the Japanese pigmentation series or to the Hokkaido "mirror" gene (p. 473). On the other hand, it does not appear from his own experiments (Goldschmidt, 1933*b*) that we are justified in regarding this character as unifactorial. On crossing European and Hokkaido races, not only did the grandparental forms segregate in F_2 , together with one of a brown shade, but there appeared also a multitude of unclassifiable types: "einer Fülle nicht klassifizierbarer Formen". Furthermore, on crossing with the dark Japanese race, classification of the segregants was not possible. The recovery of the grandparental types in the Hokkaido cross certainly suggests that very few genes are concerned, though this is less certain in the cross with the dark race from Hondo. Thus it seems to me as yet unproved that a single gene controls the dusting of white scales characteristic of the European races.

The analysis of imaginal variation in the Japanese races is, however, more definite. Those found on the main island of Japan (Hondo) are all darker than any occurring elsewhere. Machida (1924) classifies these, by the male coloration, into

black, intermediate, and brown forms. The females are hardly distinguishable. He regards them, on good evidence, as due to a series of multiple allelomorphs, with incomplete dominance of the darker over the lighter types. Goldschmidt (1933c) confirms these findings, and adds a fourth member to the series, that for the production of velvety black coloration.

The race from the island of Hokkaido is much lighter than those from the rest of Japan. The males never overlap in colour, but the females do so slightly. Machida (1924) found that this distinction was preserved unimpaired for many generations when Hokkaido insects were bred in Hondo. He crossed these with other Japanese forms, but his genetic interpretation must be superseded by that of Goldschmidt (1933c). The latter worker has shown that three genes are concerned. Of these, a produces the light ground colour. It is nearly recessive. *S* is a gene responsible for the "mirror", or patch of white scales in the centre of the wings, so often present in the Hokkaido insects. The heterozygotes are intermediate. *F*, an incomplete dominant, darkens the edges of the wings, particularly at the tips. It will be appreciated, therefore, that a cross between *Lymantria dispar* from Hokkaido and Hondo gives rise in the F_1 generation to intermediate offspring which are, however, nearer the latter type.

It may be added that the Japanese races of *L. dispar* produce certain abnormal forms, seemingly not found elsewhere. An autosomal recessive is responsible for an imperfect development of the wings, which remain soft and crumpled. It has been studied by Machida (1924). Another autosomal recessive, found repeatedly in stocks from northern Japan, obliterates the zigzag bands on the wings of both sexes, only that on the margin being left (Goldschmidt, 1927). Finally, Goldschmidt (1933c) states that a number of special arrangements of these bands, encountered in the Japanese region, have been proved uni-factorial but not further analysed.

It is not to be expected that the characters separating geographical races are often under uni-factorial control. Usually a number of genetic differences will have accumulated between them, leading to the type of segregation discussed in Section II, and illustrated by the behaviour of the English and southern French forms of *Ptychopoda seriata* there described. In such circumstances, the effects of particular genes may now and then be separable, as in the factor for speckling in that cross. More often, however, no sharp segregation can be detected. For this reason, the present discussion of the genetic differences between the imagines of geographical races is necessarily of a restricted nature. Those affecting the larvae have already been considered in Section III.

VI. PROBLEMS OF PIGMENTATION

It is to be regretted that so little is known of the chemistry of the pigments in the Lepidoptera, for a study of their constitution would throw much light on insect physiology and on the mode of action of genes. References to most of the rather meagre information on this subject, together with a survey of it, will be found in Imms (1931). Further details may be obtained from the useful account of pig-

mentation and colour in animals and plants given by Verne (1930), while mention has already been made in this article of the chlorophyll colours of the larva of *Colias philodice* and of their genetic control (p. 467). There are, however, two classes of pigments which seem to provide such fruitful material for combined genetic and chemical investigation that I wish to draw special attention to them.

It has long been known that the white and yellow pigments of the Pieridae are allied to uric acid, and indeed it has been held until recently that this substance is itself deposited in their scales. Dr B. K. Blount, to whom I am much indebted for advice on questions relating to the chemistry of pigments in the Lepidoptera, has, however, pointed out to me that this view is no longer tenable. For Wieland *et al.* (1933) have now demonstrated that the white pigment in *Pieris brassicae* L. is a special compound known as leukopterin ($C_{19}H_{19}O_{11}N_{15}$). Furthermore, Schöpf & Becker (1933) have shown that the yellow pigment of *Gonepteryx rhamni* L. is another allied substance, xanthopterin ($C_{19}H_{19}O_7N_{15}$).

Now it is a remarkable fact that there exists a very rare variety of *Pieris napi* L. (a species closely allied to *P. brassicae*) in which the white pigment is replaced by one of a brilliant yellow (var. *citronaea* Head). This is nearly as deep as the yellow pigment of the male *Gonepteryx rhamni*. The colour is equally developed in the two sexes, and both surfaces of the wings are affected. A female of this variety was bred from larvae collected at Ramelton, Co. Donegal, Ireland (Head, 1935), and a pair, together with five typical examples, were bred from a normal white female obtained in south Devon (Main, 1935). A single bright yellow *P. napi*, apparently captured in Norway, has been named *sulphurea* Schöyen. It is said to be of a different shade from *citronaea*, but it seems doubtful if they are distinct forms.

The Irish specimen mentioned above was obtained in 1909. It was successfully paired, and the stock thus originated is still in existence (Head, 1935). Though yellow specimens have repeatedly appeared in it, no genetic study of this variety has ever been made. Unfortunately, exact records are not available, for the broods have not been kept separate. Shepherd (1936) has, however, reported that he interbred typical (white) specimens from this stock, and obtained some yellow individuals among the offspring. The pairings white \times white, yellow $\delta \times$ white ϕ , and white $\delta \times$ yellow ϕ , all produced white progeny only. These were mixed and interbred, and a few yellows appeared in the succeeding generation. (He made a single pairing between two yellows, but the female damaged herself and did not lay.) From these facts it is clear, on the one hand, that the variety is genotypic and, on the other, that it cannot be a dominant (or heterozygous) form. Possibly it is a simple recessive.

Dr B. M. Hobby has, however, drawn my attention to the work of Schmidt (1913), which is very difficult to reconcile with these results. This author states that an English collector captured a bright yellow female of *Pieris napi* in Donegal. It laid fertile eggs from which he obtained 21 offspring. Of these, 14 were white (5 $\delta\delta$, 9 $\phi\phi$) and 7 were yellow (4 $\delta\delta$, 3 $\phi\phi$). Schmidt crossed one of the yellow F_1 males with a normal captured female, and reared 32 pupae. However, only 22 insects emerged from them, consisting of 5 whites (all females) and 17 yellows

(11 ♂♂, 6 ♀♀). Since the yellow form is exceedingly rare, we cannot suppose that the unknown male parent, and the white captured female used subsequently, were heterozygotes. Thus these facts seem to require the dominance of the yellow variety, in contradiction to those previously discussed. As both stocks were derived from the same locality, it is to be expected that the yellow specimens are due to the action of the same gene in each. It should be remembered, however, that this has not actually been proved. I understand that the strain derived from Mr Head's specimen is now being bred with attention to genetic requirements, and this should throw further light on the problem.

While the nature of the yellow pigment in var. *citrona* is not known, it is quite probably identical with, or closely allied to, xanthopterin. A chemical study of it is much to be desired.

The moth *Abraxas grossulariata* presents a condition superficially similar to that just described. Normally the ground colour of this species is white, but it is replaced by bright yellow in the rare variety *lutea* Cockerell. The shade approaches that of *Pieris napi* var. *citrona*. Onslow (1919a) studied this form of *Abraxas grossulariata*, and showed that it is controlled by a single factor pair, with incomplete dominance. The heterozygotes are very variable and may overlap either homozygote: selection might produce dominance here. Usually, however, they are intermediate (*semi-lutea* Raynor). It would be most interesting to know if the pigments concerned are related to those which produce such similar effects in the Pieridae.

The wings and body of many species of Arctiidae, Zygaenidae, and Noctuidae of the subfamily Catocalinae, bear markings of a characteristic pinkish-red shade. These three groups of moths are not at all closely allied, but certain facts lead me to think that this colour is produced by the same pigment, or modifications of it, in each of them.

In the first place, if the areas so pigmented in any of these species be exposed to a strong mineral acid (e.g. hydrochloric acid gas), the red colour changes to bright yellow within a minute or two; a fact apparently first noticed by Mr J. W. Tutt. Furthermore, Dr B. M. Hobby and I have independently observed that the subsequent application of an alkali (ammonia) reverses this effect, converting the yellow coloration back to the red with great rapidity.

Secondly, in nearly all these forms, genotypic varieties exist in which the red pigment is replaced by bright yellow in the living insect. This yellow colour may be superficially identical with that produced by acidifying the red pigment; but I find that, unlike that product, it cannot be converted to a red shade by treatment with ammonia. These two types of pigment, the red and the yellow, may also occur side by side in the same insect. In order to illustrate the manner in which they replace each other, we may give a short summary of their occurrence in the British species of Arctiidae and Zygaenidae known to possess them. It would take too much space to consider their distribution more widely. It will be understood that in each instance they react in the way just described.

We will discuss the Arctiidae first. The hindwings of *Callimorpha dominula* L.

are normally bright pinkish red, but several varieties exist in which they are clear yellow. In the allied *C. quadripunctaria* Poda, the hindwings may either be bright red, pale orange, or yellow: none of these forms greatly predominates. The hindwings of *Arctia caja* L. are normally red, though their shade is variable. Yellow forms occur, but I have not examined them. The red markings of *Tyria* (*Hipocrita*) *jacobaeae* L. are replaced by yellow in an exceedingly rare variety, of which I have seen a few specimens. In the female *Parasemia plantaginis* the body may either be yellow or red; the latter being the commoner. The hindwings, normally yellow, may also assume a pinkish shade; but I believe only in the red-bodied forms, and by no means in all of them. All red-bodied females also have a streak of bright red on the costa of the fore- and hindwings on the underside. Only the yellow pigment is developed in the male. In *Arctia villica* L. the ground colour of the hindwings seems always to be yellow above. The characteristic red pigment is, however, developed on the tip of the abdomen, and on the lower surface, in a bright streak along the costa of the hindwings and a short one on the costa of the forewings.

Turning to the Zygaenidae, the normal red colour of the hindwings, and of the spots or streaks on the forewings, is known to be replaced occasionally by bright yellow in each of the seven species occurring in Britain, though, in some of them, yellow specimens have only been noticed on the Continent. Such forms are very rare, and I have only been able to examine the reaction of the yellow variety of *Zygaena filipendulae* L.

The red pigment so frequently found on the hindwings of Noctuidae of the subfamily Catocalinae resembles that discussed in the foregoing groups, having the same characteristic pinkish tint. I have recently found that it also reacts in a similar way with acids and alkalis. As in the instances already mentioned, most of these species produce rare varieties in which it is replaced by bright yellow. They are known in *Catocala nupta* L. (var. *flava* Schultz), *C. promissa* Esp. (var. *ochracea* Oberth.), *C. elocata* Esp. (var. *flava* Spul.), *C. sponsa* L. (var. *desponsa* Schultz), and others. Furthermore, in many species of this genus the hindwings are normally yellow, for example *C. nymphagoga* Esp. and *C. neonympha* Esp. This yellow pigment cannot be changed to red by ammonia, nor could it in the single yellow variety of one of the red-winged species (*C. sponsa*) which I have so far been able to examine. It will be seen, therefore, that the condition in this group parallels that found in the Arctiidae and Zygaenidae.

From the foregoing body of facts, we may conclude that these red and yellow pigments are closely allied, and probably easily convertible the one into the other. Indeed the larva of *Tyria jacobaeae* is banded with yellow, and Dr B. K. Blount has suggested to me that this may represent the yellow form of the red pigment found in the adult. In this insect the gene controlling the yellow variety of the imago might therefore prevent this final conversion. (The larvae of the Zygaenidae are also yellowish.) It will, of course, be noticed that the genes determining the alternative red and yellow forms cannot merely control the hydrogen-ion concentration of the blood; for the yellow pigment, when produced genotypically, is not convertible into red by an alkali.

The genetics of this situation have so far been analysed in a few instances only.

Goldschmidt (1924*b*) describes a number of Italian races of *Callimorpha dominula* L., differing in the amount of dark pigment present (p. 480). In all of them, however, the red colour of the hindwings is replaced by yellow. Standfuss crossed a male of one of these yellow varieties (*donna* Costa) with a female from Germany having red hindwings. He found that the hindwings of the F_1 generation were orange, and that segregation in a 1 : 2 : 1 ratio took place in F_2 . On the other hand, another variety with yellow hindwings has long been bred in England by Mr L. W. Newman, and few large collections are without specimens of it. This is var. *lutescens* Oberth. (often wrongly called *rossica* Kol.). Cockayne (1928*b*) records that this is recessive, but that ova cannot be obtained by mating two homozygotes: Mr Newman has preserved his stock by crossing heterozygous reds. Since the Italian races in question all have yellow hindwings, it is certain that no such barrier exists in them. It would be extremely interesting to know if different genes are involved, or the same gene in different gene complexes.

The genetics of *Callimorpha quadripunctaria* Poda have not been studied but, in the absence of evidence, it is reasonable to suggest that a single factor pair may control the development of red and yellow pigment in the hindwings, orange specimens being the heterozygotes. Cockayne (1925*a*) regards the females of *Parasemia plantaginis* L. as falling into three groups: (1) with red hindwings and abdomen, (2) with red hindwings and yellow abdomen, and (3) with yellow hindwings and abdomen.¹ He bred from a female of type-2 and obtained a brood including 30 female offspring, of which 26 were of type-2 and 4 were of type-3. A simple segregation is at least suggested here. It will be noticed that this species provides an exception to the statement of Goldschmidt (1933*c*, p. 165) that wing and body colour seem to be determined simultaneously in all Lepidoptera.

Unfortunately nothing is known of the genetics of the Catocalinae; we have, however, a little information on the inheritance of the red and yellow pigments in the Zygaenidae. At the time of his death, Onslow was working on *Zygaena filipendulae* L. He had reported that the yellow form appeared to be a simple recessive (Bateson *et al.* 1923), but that larger numbers were required to prove the point. Mr L. W. Newman has bred this variety for many years, and he has kindly provided me with a summary of his results, which confirm Onslow's suggestion. It is also corroborated by the findings of Grosvenor (1932-3). Bovey (1936) has studied the genetics of *Zygaena ephialtes* L., inhabiting continental Europe, and his data show that the yellow forms are recessive to the red in that species also.

Dr B. K. Blount has suggested to me that the colour reactions and solubility of these pigments make it probable that they are to be classed as "pterins". It is to be hoped that they will be studied more fully, both chemically and genetically, in the future. No doubt it will be found that they are not restricted to the three groups mentioned here;² indeed they may prove to be of rather wide occurrence in the Lepidoptera, and perhaps outside that order.

¹ I have, however, seen a few specimens which it would be rather hard to classify on this basis.

² It may be added that the red colour on the body of *Lymantria monacha*, which is replaced by yellow in var. *flavoabdominalis* Schultz, is due to a pigment of this type. (See also the footnote on p. 497.)

VII. MELANISM

The occurrence in the Lepidoptera of blackish or "melanic" forms among species normally of paler coloration presents one of the most interesting aspects of variation in this order. Such aberrations may be phenotypic or genotypic in origin, but here we are concerned only with the latter. For the purpose of discussion they may conveniently be grouped under three headings. First, those instances in which melanics occur sporadically, as rarities. Secondly, those in which they constitute local races, comprising a considerable proportion, or the whole, of the population in regions unaffected by industrial conditions. Thirdly, the interesting phenomenon of industrial melanism itself.

I use the term sporadic melanism to include those blackish varieties which occur so rarely that we have no reason to regard them as polymorphic forms in the species concerned (p. 489). That is to say, they are presumably subject to adverse selection, but are maintained at a low level in the population by recurrent mutation. It is clear that when the melanic variety of a species is well established in one district, it will probably be found as a rarity throughout its whole range. In fact we are here dealing with such forms as have nowhere succeeded in establishing themselves permanently.

Instances of sporadic melanism are of very wide occurrence, especially in moths, but they are known also in many butterflies; for example, in *Papilio machaon* L., *Melanargia galatea* L., and in a number of the Nymphalidae. Owing to their rarity, they have not often been studied genetically; for, when obtained, collectors are inclined to keep them as cabinet specimens, instead of breeding from them. However, it is known that melanism behaves as a recessive both in *Ectropis* (*Tephrosia*) *bistortata* Goeze (Harrison & Garrett, 1926) and in *Selenia bilunaria* Esp. (Harrison, 1928). Black specimens of these species have very seldom been found in nature. Cockayne (1925*b*) bred from a female of the melanic form *obscura* Tutt, of *Meristis trigrammica* Hufn. He obtained 13 offspring, 12 being normal and 1 dark, but lighter than the true *obscura*. From this he concludes that the form may be a simple recessive, but the possibility of some multi-factorial basis cannot be excluded. The melanic variety of *Ephestia kühniella*¹ is also inherited as a simple recessive (Kuhn & Henke, 1929-36).

Black specimens have been recorded in several species of Zygaenidae, but only on a few occasions. They have occurred repeatedly, though very rarely, in a colony of *Zygaena trifolii* Esp. in north Sussex. Gosvenor (1926-7) showed that they are recessive to the typical form, and noticed a tendency to preferential mating in his stocks: the black males select normal females, and the red males select black females.

The extremely uncommon black variety (*unicolor* Tutt) of the Sphingid *Sphinx pinastri* L. has been bred in somewhat remarkable circumstances (Cockayne, 1926).

¹ A number of other genes has been successfully analysed in this species. As they have appeared and been studied in experimental cultures, they hardly fall within the scope of this article. Furthermore, a complete account of them, together with a summary, has been compiled by Kuhn & Henke (1929-36), consequently they have been omitted here.

The species is one which, though still rare, has increased in certain districts of England in recent years. A female of a new form, *albicolor* Cockayne, was found in Suffolk by Mr M. Mactaggart. It has the ground colour of the body creamy white, the wings being of this shade also, but rather thickly dusted with brown scales, giving them a light brown tint. When discovered, it was paired with a normal male, both specimens being newly emerged and, presumably, members of the same brood. They produced an F_1 consisting of 7 typical specimens and 6 of another new form, *albescens* Cockayne. This resembles *albicolor* but lacks the dusting of brown scales, being entirely cream coloured. Two of the typical specimens were interbred to give an F_2 generation comprising 26 normal *pinastri*, 11 *albescens*, 11 *unicolor*, and 1 *albicolor*. Thus, as Cockayne points out, it is clear that both *albescens* and *unicolor* are single recessives, and that *albicolor* is the double recessive combination of them. It is therefore surprising that the latter variety was actually found in nature. The male with which it was paired when discovered must have been heterozygous for the *albescens* gene.

We may now consider those species in which melanic forms occur regularly, in certain districts at least. These are, in reality, examples of polymorphism, a phenomenon to be defined and discussed in Section VIII. It is, however, convenient to keep the various types of melanism together, for treatment as a single problem.

A dark race of *Triphaena comes* Hb. (*orbona* Hufn.), known as *curtisii* Newm., is found not uncommonly in north-east Scotland. Bacot (1905) showed that it is inherited as a dominant, the heterozygotes being, however, somewhat lighter in colour than the homozygotes. The Geometrid *Lygris populata* L. is normally light yellow, but a dark purplish brown variety (*musauaria* Fr.) is not rare on moors and high ground in some localities. Walther (1927) has demonstrated that it behaves as a single dominant. The dark form (*obscura* Fuchs) of *Ptychopoda eburnata* Wocke (= *Acidalia contiguaria* Hb.) is also a dominant (Bowater, 1914). Barrett (1902), in his work *The Lepidoptera of the British Isles*, describes this as "a constantly recurring form or permanent variety, found sometimes at greater elevations on the mountains". The black varieties *Boarmia consonaria* Hb. var. *nigra* Banks, *B. punctinalis* Scop. (*consortaria* Fb.) var. *consobrinaria* Bkh., and *B. extersaria* Hb. (*luridata* Bkh.) var. *cornelsenii* P. Hoff. (see p. 487) are each dominant to their normal paler forms (Onslow, 1919*b*, 1920*a*; Harrison, 1932*a*; Hasebroek, 1934). All of them first appeared in Kentish woods at a distance from any industrial areas (Onslow, 1920*a*). Similarly, the melanic variety of *B. ribeata* Cl. (*abietaria* Schiff.) was first reported from country districts, in Surrey and the New Forest. Onslow (1920*b*) has shown that it also is dominant to the typical form.

I find it rather difficult to determine the status of the melanic varieties of *Spilosoma lubricipeda* L., which comprise var. *xatima* Stoll. and its modifications. Seitz (1913) remarks: "The most important localities in which these aberrations are found are the coasts of the Channel and the Low German coast with its islands." South (1907) states that this form was at one time known only from Heligoland. In England it has largely been bred from the Barnsley (Yorkshire) stock of Mr Harrison, but this seems to have originated from a single specimen

which emerged from a batch of mixed English pupae. Thus, although rare, the *zatima* forms appear to be characteristic of certain districts, but not especially of industrial ones. Consequently they may appropriately be mentioned at this point. The extensive series of *zatima* forms produced by collectors through interbreeding and outcrossing have been analysed successfully by Federley (1920). He has shown that the heavy melanic development of *zatima* is controlled by a single factor pair, having a somewhat less marked effect in the heterozygotes than in the homozygotes. Multiple factors influence the expression of this gene, their effect being greater in heterozygous than in homozygous *zatima*, and least of all in the normal *lubricipeda*.

Several factors are also concerned in producing the dark races of the Arctiid moth *Callimorpha dominula*, but each exerts a major control, instead of a single one acting as the chief determinant with the others having a subordinate effect, as in the last instance. In northern Italy occurs var. *italica* Standf., with the whitish spots on the forewings larger than normal. In Tuscany and Calabria a melanic form *persona* Hb. is found, in which these spots are nearly obsolete, and the ground colour of the hindwings is largely obscured by the confluence of the black markings. Individuals in which this fusion is so complete that only an isolated spot of the ground colour is preserved on the hindwings are generally called *donna*¹ by collectors. In a locality in Abruzzi, near the Grand Sasso, all these forms, from pale *italica* up to the darkest *donna*, fly freely together. Standfuss crossed a male *donna* with a normal German female. The F_1 had dark markings of intermediate degree (and an orange ground colour to the hindwings, see p. 477). These he called *romanovi*. They were interbred to produce an F_2 , which showed that two pairs of darkening factors **A** and **B**, with intermediate heterozygotes, were concerned. Homozygous *donna* has the constitution **AABB**, while lighter *donna* has three darkening factors. *Persona* carries **AaBb**, light *persona* has one darkening factor only, and the normal *dominula* has none (Goldschmidt, 1924b). These Italian forms have the ground colour of the hindwings yellow instead of the usual red. This character is inherited independently of the dark coloration, being uni-factorial with an intermediate heterozygote, as already described on p. 477.

It would be interesting to know if the darkening factors **A** and **B** bear any relation to the one discussed by Cockayne (1928b). This is from an English locality. It produces a variety, described by him as *bimacula*, in which all the forewing spots are obscured except the two at the base, while the red hindwings are heavily marked with black. The heterozygotes are intermediate; the central spot on the forewings being small or absent, the others reduced, and the black markings on the hindwings are increased (var. *medionigra* Cockayne).

Polymorphism, whether involving melanism (as in *C. dominula*) or not, has been investigated in a number of species (Section VIII). It is generally controlled by genes at different loci, interacting to produce their effects. However, that studied by Standfuss (1910) on the melanic forms of the moth *Agria tau* L. provides an illustration of a somewhat different mechanism, depending upon multiple allelomorphism. The species is European, and two forms, both darker than the typical

¹ If this name has validity, it is the *donna* of Costa, not of Esper.

tau, occur as a small percentage of the total population in nature (2 or 3 per cent each), but Hasebroek (1934) states that they have recently become much commoner in manufacturing districts. One of them, *melaina* Gross., has a black male and a blackish brown female, but the wings are lighter underneath. The other, *fere-nigra* Th. Mieg., is black above, with a yellowish median area, but is darker than *melaina* below. Each is described as dominant to the normal *tau*, but the homozygotes are darker than the heterozygotes. When either of them is obtained in nature and crossed with *tau*, segregation in a 1 : 1 ratio takes place among the offspring. However, a cross between wild *melaina* and *fere-nigra* produced all three forms, together with a new one, *weismanni* Standf., which combined their characteristics. These segregated in approximately equal numbers (10 *tau*, 11 *melaina*, 15 *fere-nigra*, 11 *weismanni*). The cross *weismanni* × *tau* gives *melaina* and *fere-nigra* only, in equal numbers. *Weismanni* mated *inter se* produce *melaina*, *weismanni*, and *fere-nigra* in a 1 : 2 : 1 ratio. The *melaina* and *fere-nigra* so obtained are all homozygotes, for they give *weismanni* only when crossed together. It thus appears that the genes controlling *melaina* and *fere-nigra* are multiple allelomorphs, and that a single dose of each produces *weismanni*. That is to say, *tau* = mm , *melaina* = Mm and extreme *melaina* = MM , *fere-nigra* = M^Fm and extreme *fere-nigra* = M^FM^F , while *weismanni* = MM^F .

The problems presented by industrial melanism are of great interest and importance. Many different species of moths have become black in industrial areas in England and continental Europe, the change affecting either the whole population or a large proportion of it. From these districts such forms have in some instances spread into the surrounding country, gradually being replaced by typical specimens as the distance from their centre of origin increases. This process has been observed in very distinct groups. It has taken place almost entirely during the last 80 years and, in many of the species, even within living memory; furthermore, it is still actively proceeding. Indeed it represents one of the few evolutionary modifications ever actually witnessed in nature, and it is certainly the most considerable of them.

Students will find accounts of the spread of melanic moths in the manufacturing areas of Britain in the papers of Doncaster (1906), Harrison (1920*a*) and Adkin (1925-6), and a summary of it on the Continent may be obtained from Gerschler (1915), Walther (1927) and Hasebroek (1934). Before considering the possible explanations of this phenomenon, we will review those instances in which it has been studied genetically. It may be added that the same process has affected many larvae (Harrison, 1932*b*), but so far it has been analysed in very few of them. (For an account of it see Section III.)

It has already been mentioned that the melanism of *Oporinia dilutata* has a multi-factorial basis (p. 463). Dark specimens, varying in degree, are found in a number of industrial districts, especially in northern England. In *Lymantria monacha* the long series of forms, from normal to completely black (var. *atra* Linst.), is also multi-factorial. It has been the subject of an admirable study by Goldschmidt (1921), who has demonstrated that it is produced by the interaction of three factor pairs. The most important of these is a sex-linked dominant (C). This, by itself,

produces a suffusion of black pigment on the forewings, spreading from the margin and central bands, and it completely blackens the hindwings. Secondly, an autosomal dominant (**B**) induces an increase of pigment between and around the central bands, and a general greying of the males when homozygous. Its effects are less extreme in the heterozygotes than in the homozygotes, and in the females than in the males. The blackest specimens always contain the genes **C** and **B**. Another autosomal dominant (**A**) increases the effect of the two other factors and, by itself, intensifies the pattern. The proportion of melanic forms of this moth have greatly increased in Europe during the last 60 years.

There exists a blackish form of *L. dispar* which corresponds with the intermediate stages of darkening in *L. monacha*, but it is inherited as an autosomal recessive (Klaat, 1928). The two well-known dark forms of *Abraxas grossulariata* are both recessives also (Onslow, 1921*a*). They are very uncommon. The more extreme of them, *varleyata* Porritt, has been captured in the neighbourhood of Huddersfield on a number of occasions. The whole area of the wings, except the base of the inferiors and, generally, a narrow band on the superiors, is deep black. In *hazeleighensis* Raynor the black pigment is not localized in spots, but spreads over most of the forewings. Having a somewhat similar, but less extreme, effect than *varleyata*, it is hardly detectable in the latter form. However, the two genes are often present together and, though they are described as segregating independently, there may be linkage between them. It is very curious that *varleyata* is recessive to the pale form *dohrnii* (p. 470), but the double recessive combination of them is said to produce var. *exquisita* Raynor. It appears that the variety known as *lunulata* Raynor, which is heavily marked with black, and has little or no yellow in the band, is dominant to *varleyata*. Cockayne (1928*a*) bred from a pair of *lunulata*, and the offspring consisted of 75 per cent of that form and 25 per cent of *varleyata*.

A series of multiple allelomorphs control the ground colour of the Tortricid moth *Acalla comariana* (Fryer, 1928). The gene **B**, producing the normal brown shade, is dominant both to that for marbled (**B**₁) and grey (**B**₂) coloration. Fryer (1931) concludes that the gene controlling the melanic form *fuscana* Sheldon, from Lancashire, is also a member of this series (**B**₃). It has a slight effect in the presence of **B**₁ and **B**₂, and moths of the constitution **BB**₃ are almost intermediate.

An intensely black variety (*thompsoni* Arkle) of the Noctuid *Aplecta nebulosa* Hufn. occurs in Cheshire and other districts within the manufacturing region of northern England. This is an instance of a single factor difference without dominance. The intermediate heterozygotes are known as *robsoni* Collins (Bowater, 1914).

A number of the melanic varieties found in industrial areas are dominant to their normal forms. Gerschler (1915) has made an interesting study of the black variety (*albigensis* Warn.) of *Palimpsestis* or *Fb.*, in the neighbourhood of Hamburg. He records that it was very rare in 1904, while by 1911-12 about 90-95 per cent of the larvae collected in this area produced it. The form is a simple dominant. Of much ecological interest also are the records of Walther (1927) on *Monima* (*Taenio-campa*) *populi* Ström. (*populeti* F.). He studied its melanic aberration (*nigra* Tutt) at Wilsdruff, where it first appeared in 1917, and reports that the proportion of black

specimens is greatest in those years in which the species is commonest. Indeed he is led to remark: "Man hat den Eindruck, dass besonders günstige Entwicklungsverhältnisse die schwarze Form leichter entstehen lassen." An instance has been studied in the Lepidoptera in which a rapid increase in numbers was accompanied by increased variability (Ford & Ford, 1930), and I have elsewhere discussed the evolutionary possibilities of such fluctuations (Ford, 1934). This black form of *M. populi* is uni-factorial and dominant. I am not quite certain if it is to be treated as an example of industrial melanism, for the district of Wilsdruff can hardly be regarded as a manufacturing area. On the other hand, the town is situated only about 12 km. distant from Meissen, with its great porcelain works. Walther also states that a variety of this moth, which he calls *atropunctata*, is inherited as a dominant. No such form is mentioned by Seitz, and Mr W. H. T. Tams, from whom I made enquiries, has kindly informed me that he can find no record of it. It is probable therefore that it has been incorrectly named.

A melanic form (*nigra* Prout) of *Gonodontis bidentata* Cl. has become common in the manufacturing districts of northern England. Onslow (1921*a, b*) incorrectly states that it is a recessive, but this is an error. Very thorough work by Bowater (1914), which involved the breeding of thousands of specimens, had clearly proved it to be a simple dominant.

I am indebted to Mr W. Buckley for most kindly allowing me to quote the results of his breeding experiments on *Phigalia pedaria* F. He found two specimens of the black var. *monacharia* Stgr. paired near Barnsley. They produced an F_1 of 6 normal (4 ♂♂, 2 ♀♀) and 9 black (6 ♂♂, 3 ♀♀) offspring. He paired two of the *monacharia* so obtained to produce an F_2 consisting of 9 black and 2 typical specimens. On another occasion, the mating black \times normal gave rise to an F_1 of 10 normals (6 ♂♂, 4 ♀♀) and 8 melanics (6 ♂♂, 2 ♀♀). It thus appears that *monacharia* is a simple dominant. This form has now spread rather widely in the manufacturing areas of northern England.

As is well known, the earliest instance of industrial melanism was provided by *Biston betularia* L. The black form *carbonaria* Jordan (*doubledayaria* Mill.) seems to have been detected for the first time in 1850 at Manchester. It is entirely black, except for a white dot at the base of the forewings, and is inherited as a simple dominant (Lemche, 1931). It has completely replaced the typical form in the industrial areas in England and elsewhere, and has spread from them into the surrounding country districts. A much less common form (*insularia* Th. Mieg) has the normal black speckling greatly extended, largely, but not completely, obscuring the white ground colour. Indeed it looks as if it were a heterozygote between *carbonaria* and *betularia*. But this is not so; for it is controlled by a separate dominant gene (Lemche, 1931). Its presence cannot be recognized in the wholly black *carbonaria* form.

A brownish variety (*fuscata* Tutt) of *Hemerophila abruptaria* Thnbg. has become frequent in the London district. It is inherited as a single dominant (Onslow, 1921*c*). So also is the melanic variety (*rebeli* Aigner) of *Boarmia rhomboidaria*. I am much indebted to Mr H. B. Williams for placing at my disposal the

records of his detailed breeding experiments on this form (see also p. 471). They amplify his previous account of its genetics (Williams, 1932-3). He finds that certain discrepancies occur in the ratios expected on the assumption of its simple dominance. However, they seem always due to an excess of melanics, and are doubtless to be explained by the fact, which he noticed, that these are hardier than the typical specimens. *Rebeli* occurs chiefly in Kent, but another dark variety, *perfumaria* Newman, has been found occasionally throughout the range of the species. Though much darker than normal, this does not approach the black of *rebeli*, from which it is easily distinguished. Its genetics appear to be unknown, but Williams received ova, said to be from a black strain, which produced *perfumaria* only for three generations, the second of which comprised 93 specimens. Eggs laid by a female of this form caught in Ayrshire produced 13 offspring all *perfumaria*, which may therefore be a dominant. A male of this family crossed with a female *rebeli* produced 3 progeny only: 2 *rebeli* and 1 *perfumaria*.

The black form of *Boarmia repandata* L., known as *nigricata* Fuchs, has become increasingly common in industrial areas, while the pale variety *conversaria* Hbn. is now extremely rare in such districts, even in localities where it was found in the past. Walther (1927) has shown that both are dominant to the normal *repandata*, but that the effect of the *conversaria* gene cannot be detected in the *nigricata* form. Tonge (1915-16) had bred from a *conversaria* female fertilized in the open, and had obtained an F_1 of 30 *repandata* (1♂, 29♀♀) and 39 *conversaria* (0♂, 39♀♀). This extraordinary family must be the result of some sexual abnormality. Indeed all-female families have several times been reported in the Lepidoptera. However, this one is quite consistent with the dominance of *conversaria*.

Ectropis crepuscularia Hbn. provides one of the best-known instances of industrial melanism. The whole normal population of the species has been replaced by the deep greyish variety *delamerensis* B. White in many manufacturing districts in England and continental Europe. Harrison & Garrett (1926) have shown that it is inherited as a dominant. It will be recalled that the rare melanic variety of the very closely allied *E. bistortata* behaves as a recessive (p. 478). It differs in appearance from *delamerensis* in being deep black, instead of greyish.

Harrison has obtained very interesting results by hybridizing these two species. In the cross melanic *E. bistortata* × normal *E. crepuscularia* the melanism remains recessive, as in the former species (Harrison, 1926). The results of the reverse cross (normal *E. bistortata* × melanic *E. crepuscularia*) are somewhat inconsistent. In one set of experiments, either all or half the F_1 individuals were melanics (depending on whether the melanic *E. crepuscularia* parent was homozygous or heterozygous), while a complete breakdown in dominance occurred in F_2 , giving an unclassifiable range from pale to dark forms (Harrison, 1920b). In a second, and similar, experiment the melanism brought in from the *E. crepuscularia* parent retained its dominance, with no great variation in F_2 . However, a streaked form appeared, which seems to be a multiple allelomorph at the locus of the *delamerensis* gene. This also behaved as a dominant to the normal form (Harrison, 1923). In these tests the melanic *E. crepuscularia* were derived from a number of different localities, but

none were of the same stock which had given the greater F_2 variation in the previous work. All the specimens were of British origin. The same type of cross (normal *E. bistortata* \times melanic *E. crepuscularia*), in which the *E. bistortata* were English and the *E. crepuscularia* continental, gave similar results to those last described; the melanism remaining a complete dominant (Harrison, 1927). Finally, Harrison (1932a) succeeded in obtaining the cross melanic *E. bistortata* \times melanic *E. crepuscularia*. The F_1 were all grey melanics (*E. crepuscularia* type), and F_2 segregated into grey melanics (*E. crepuscularia* type), non-melanic individuals, and black melanics (*E. bistortata* type), in a 9 : 3 : 4 ratio (337 : 113 : 153). Harrison's comments on these results are expressed in rather confusing terms, for he concludes that "in *T. [= E.] crepuscularia* melanism is epistatic not dominant to type, i.e. that homozygous melanics carry 'type' likewise". Actually the experiment simply demonstrates that melanism in these two species is due to different genes, which retain their respective dominant and recessive effects. The meaning of his further deduction, that melanism is probably epistatic in six other species in which it has been deemed a dominant, is not altogether clear.

The dominance relationships revealed by this work present certain difficulties. Melanism derived from *E. bistortata* retains its recessiveness in F_1 and F_2 both in crosses with normal English, and melanic continental, *E. crepuscularia*. Since these two species are very nearly related, it is possible that this is due to a gene to which both are accustomed. It may well have reached a recessive condition in each, owing to adverse selection (not necessarily in respect of its effect on coloration). If so, it has not yet been detected in *E. crepuscularia*, but it has only been found in *E. bistortata* on a few occasions.

The behaviour of the melanism derived from *E. crepuscularia* is rather more peculiar. In one instance its dominance broke down in the F_2 resulting from the cross, but in a number of others it was retained. Doubtless these two closely allied species of *Ectropis* have a considerable part of their gene complex in common, but the *E. bistortata* share of the hybrid will not have been selected to procure the dominance of the *delamerensis* factor. Since, however, it was operating throughout in a genic environment not less than half its own, we are not justified in concluding that dominance was reached by other means than selection of the gene complex. However the point is one which could be resolved by the experiment of back-crossing *delamerensis* to *E. bistortata* for (say) five generations. (This was the number adopted by Fisher (1935) in his well-known work on dominance in poultry.) By that time, 31 out of every 32 genes with which the *delamerensis* factor has to interact would be derived from the species unaccustomed to it. A comparison between its homozygous and heterozygous manifestation in such a genic background should certainly decide whether the dominance of *delamerensis* is a property of the factor controlling it, or of the reaction of the internal environment to that factor. It would be worth while to conduct such a test. The present survey shows that simple recessives are not included among those melanic varieties which have largely replaced the typical form of their species in certain areas. This is in harmony with the view that the recessive state is reached by counter-selection.

The spread of melanism among the Lepidoptera of industrial areas has aroused widespread interest, and several theories have been advanced to account for it. The most obvious explanation is the direct action of selection in favour of dark forms. It has been held that these will be less conspicuous, and therefore at an advantage, in manufacturing districts where the countryside is blackened by smoke. Personally I believe this to be a factor in the increase of melanism in some species. It has frequently been reported to me, and I have myself observed, how much more inconspicuous are the dark than the typical insects in such areas: while the reverse is certainly true in many situations outside them. On the other hand, this view fails to account for all the facts. It seems that in some species the selective advantage due to colour is too slight to bring about the rapid change with which we are dealing. Thus Harrison (1920*b*) obtained records in the morning and evening of the numbers of light and dark *Polia chi* on three differently coloured walls. His observations were on a large scale, comprising many thousands of specimens. He reports that not only did no differential elimination of light and dark forms occur, but that it was most exceptional for even a single specimen to disappear during the course of the day.

Harrison himself holds that the melanism is induced by salts of lead and manganese contaminating the food plants of the larvae. These substances occur in the film of soot on the leaves in industrial areas and, as he was able to show, they are taken up by the tissues of the plants. On this view, lead and manganese salts act as agents in inducing the mutation of the genes controlling melanism; and he has conducted many experiments to demonstrate it (Harrison & Garrett, 1926; Harrison, 1928, 1935). For this purpose he has principally used *Selenia bilunaria* and *Ectropis bistortata*, feeding stocks from non-industrial areas for several generations on food containing these salts. Melanism, which was inherited in normal fashion, appeared in the broods feeding on the treated plants, but not among the controls kept on uncontaminated leaves.

This work has been subject to much criticism. Hughes (1932) and Thomsen & Lemche (1933) have failed to confirm it on *Selenia bilunaria*, using very large numbers, a fact which Harrison (1935) explained by pointing out that there had been a heavy death rate among the larvae. This, he holds, had resulted in the differential elimination of the melanics which, in that species, are less hardy than the normal form. Fisher (1933) has drawn attention to the enormous mutation rate required by Harrison's theory: many times larger than that attained by X-ray treatment in the *Drosophila* work. Indeed, Fisher points out that Harrison's data do not exclude the possibility that the black forms, which undoubtedly appeared in his treated stocks, were due to segregation in material originally containing heterozygotes.

At their first occurrence in the experimental strains, the melanics did not appear in simple Mendelian ratios, but in much smaller proportions. In subsequent breeding work, however, their approximation to Mendelian expectation was very close. This constituted rather good evidence in favour of mutation. Unfortunately it is no longer of value in face of Harrison's own statement that the melanics

are less hardy than the normals in the species which he investigated. This fact might well account for their smaller numbers in the broods in which they first appeared.

In my opinion, melanic forms have spread in industrial areas owing, primarily, to selection for characters other than colour. The action of the genes producing melanism as one of their effects may sometimes give the organism a physiological advantage. That such favourable factors have not become widely established may be due to the handicap of black coloration which, in normal circumstances, would render some species very conspicuous. On the other hand, melanism, as such, may at least be no longer a drawback in the blackened countryside of many manufacturing districts, in which, furthermore, the number of predators may be reduced. Here, then, the insects may be able to avail themselves of the other benefits conferred by these genes.

Such a view is not without confirmation. In a number of instances it has actually been demonstrated that the melanic varieties are hardier than the normal form. This has been shown by Onslow (1920*a, b*) in *Boarmia consonaria*, *B. punctinalis*, and *B. ribeata*. Now, though all three have a fairly wide distribution in England, black specimens are almost entirely confined to localities in Kent, Surrey, or Hampshire. That these more viable forms have not spread in the population is a fact deserving of careful attention. I think it probable that they would have done so but for their colour, and that they would in fact have proved a success in industrial areas, had these moths occurred in them. Indeed it is striking that a melanic form, *humperti* Hum., of *B. punctinalis*, very similar to that found in England, has thoroughly established itself where the species occurs in industrial areas in Germany (Hasebroek, 1934). So also has var. *cornelsenii* of *B. extersaria*, which has not replaced the normal form in rural England although not unknown there (p. 479).

Williams (1932-3) has found that var. *rebeli* of *Boarmia rhomboidaria* (pp. 483-4) is also hardier than the typical specimens. Though the species has a wide range, this melanic form is almost restricted to Kent. It would be most interesting to introduce specimens of it into distant manufacturing and rural districts respectively, and to compare its spread in them.

Unfortunately, few data are available on the relative viability of the melanic and paler forms in those species in which the former have replaced the latter in smoky neighbourhoods; such observations are greatly to be desired. However, Onslow (1921*c*) states that the melanics are the hardier in *Hemerophila abruptaria*, the dark form of which is now common in the London district. Bowater (1914) has definitely established the same fact in *Gonodontis bidentata*. He further records that the black specimens can emerge earlier in the year, and at a lower temperature, than those of normal coloration, which they have superseded in the manufacturing districts of the north. But, in spite of their favourable qualities, they have not done so in other parts of the insect's range, farther north or south.

When the disadvantage of black coloration in normal country is very nearly balanced by a physiological advantage conferred by the gene producing it, and well outweighed by it in manufacturing districts, we may expect a considerable spread

of melanics into non-polluted areas since the advent of industrial melanism. This has, in fact, been observed in several species.

It has already been mentioned that the melanics are *less* hardy than the normal form in *Selenia bilunaria* and *Ectropis bistortata*. But as they are only known as rare aberrations in these species, no difficulty arises here.¹

Finally another possibility, involving a somewhat different selective basis, must at least be mentioned. Attention has already been drawn to Harrison's work (p. 486), showing that plants in industrial areas are often covered with a fine deposit of soot containing salts of lead and manganese, and that these substances may be present in their tissues. The physiological action of the genes producing melanism as one of their effects may occasionally give the organism an advantage compared with the normal specimens when feeding on contaminated food. Thus even black forms, which are less viable than the typical insects in unpolluted country, might sometimes prove the hardier in manufacturing districts.

No direct evidence of such a condition exists, but somewhat analogous situations have been encountered. For example, Spencer (1932) showed that though the vestigial winged mutant of *Drosophila melanogaster* is at a disadvantage compared with the wild type in normal circumstances, it is the longer lived in the absence of food and moisture. If such an effect as that now under discussion really contributes any considerable share to the spread of melanism in manufacturing areas, which I rather doubt, it remains to be explained why genes having an advantage when the food is contaminated should especially tend to produce melanism as an incidental effect. However, it may be that the particular type of metabolism resulting in melanin formation has certain advantages in the presence of the lead and manganese salts so frequent in soot. That it does not always do so is proved by the poor viability of the *Selenia bilunaria* and *Ectropis bistortata* melanics on treated food. It will be noticed that the difficulty mentioned here does not affect the alternative hypothesis. For all genes conferring greater viability will in fact have spread through the species, except those entailing some countervailing disadvantage such as melanism may be in non-industrial areas.

Apart from localities blackened by smoke, there is also a general tendency for moths to be darker in northern Britain. This is perhaps due to the fact that light-coloured geological formations are uncommon in this region, while the increased moisture has a darkening effect on rock and other surfaces. The latter consideration may help to explain a similar slight effect sometimes observed along the coast. But the colour of the rock is often a factor here also: as on the coast of Devon and Cornwall where dark sandstones are prevalent, and some species of moths are of a deeper shade than usual. It should be noticed that, in order to explain this tendency, we cannot invoke the induction of melanism by salts taken

¹ Since writing these passages, I have read the account of Robson & Richards (1936), who refer briefly to industrial melanism (pp. 213-5). They draw attention to the fact that some melanics are hardier than the normal form, and rightly remark that this discovery should be followed up. However, no explanation of industrial melanism can be regarded as adequate which does not take into account the significant fact that melanic forms possessing marked physiological advantages have none the less been unable to establish themselves outside industrial areas.

up in the food plants in coastal districts. For moths inhabiting the seaboard in the chalk area are, on the whole, markedly paler than the average of the species.

In short, the spread of melanism in industrial neighbourhoods is here attributed not to an induction of mutation by the contaminated food, but to the selection of genes normally present as rarities in the population. When these combine melanin production with a physiological advantage, a proved fact in some instances, it is suggested that they have in the past been unable to establish themselves owing to counter-selection for colour. This is supported by the existence of melanic varieties actually hardier than the ordinary form which, none the less, they do not supplant in unpolluted country. It is further suggested that black coloration is no longer a disadvantage in manufacturing districts. Thus the various species can there take advantage of any favourable physiological effects of the genes producing it, which consequently spread in such areas.

VIII. POLYMORPHISM

Polymorphism is the existence, in the same habitat, of two or more forms of a species in stable equilibrium. As pointed out by Fisher (1927), this must always imply that a balance of selective agencies maintains them in definite and optimum proportions, departure from which constitutes a disadvantage. Were this not so, the most favoured variety would spread through the species and establish uniformity. The component tending to increase variability in this system is often genotypic; but it may have a different basis, as in the social Hymenoptera in which it depends partly upon varying the food supplied to the larvae. That tending, on the other hand, to decrease it, may either be genetic also or ecological. Both these methods of checking the variance are exemplified in the Lepidoptera. The first of them becomes effective when a variety enjoying some advantage is less viable as a homozygote than as a heterozygote. The second is attained in the mimicry of a more protected by a less protected species. The gain from this wanes, and is converted into a disadvantage, as the numbers of a mimic rise relative to those of its model. Other genetic or ecological situations may of course be concerned in the balance of polymorphism, and often its basis is unknown. It is convenient, therefore, to bring together the various types which do not seem to involve mimicry; so separating them from mimetic polymorphism, the mechanism of which is probably more uniform. As already mentioned, certain melanic forms appear to be maintained on a polymorphic basis, but these have been described in the last section (pp. 479-81).

(1) *Non-mimetic polymorphism*

The males of the butterfly *Argynnis paphia* L. are always of a rich brown colour, with black markings. The females, however, are dimorphic. One form (*paphia*) much resembles the males, while the ground colour of the other (*valexina* Esp.) is dull green. Goldschmidt & Fischer (1922) have shown that the latter is due to the action of a single dominant factor, which is autosomal and sex-controlled. It is linked with a recessive lethal throughout the greater part of the species' range. This

makes it impossible to obtain the homozygous dominant, and leads to segregation in a 2 : 1 ratio in the F_2 generation. Consequently *valexina* is generally rare, though it forms a definite element in the population, of perhaps 5-15 per cent, in a few districts, for example the New Forest in England. During the course of their work, Goldschmidt & Fischer found it possible to eliminate the lethal. This enabled them to produce families in which all the females were *valexina*, and to obtain the expected 3 : 1 ratio in F_2 . However, the factor seems to be free from its lethal association in the eastern part of the Palaearctic region, for here nearly all the females are *valexina*.

A situation genetically similar is widespread in the genus *Colias*. In many of the species the males are monomorphic and bright yellow, while the females may either be very similar to them or whitish. The latter form is nearly always the rarer. It is inherited as an autosomal sex-controlled dominant in every instance so far analysed, and it is generally linked with a recessive lethal. This dimorphism has been studied most thoroughly in the North American *C. philodice* Godt. by Gerould (1923). He too was able to eliminate the lethal from his stocks, so obtaining a 3 : 1 instead of a 2 : 1 ratio in F_2 , and rearing broods in which all the females were white. He found that the lethal was brought in again when such stocks were crossed with wild specimens. In nature, the white females (*alba* Stgr.) generally vary from 4 to 20 per cent. It is said, however, that they are commoner than the yellows in a restricted area in New England. Probably they are free from the lethal in that district. Although this gene is normally without effect in the male, occasional white specimens of this sex have been reported by collectors. Unfortunately it has not yet been possible to study them genetically.

Gerould (1923) has conducted similar studies on two other species of North American *Colias*. In *C. eurytheme* Bsd. the white female form (*albina* Seitz) is subject to the same type of control as that in *C. philodice*, and indeed appears to be due to an identical gene. The two species can be crossed, and it may be introduced into the hybrid from either. Not only is its general influence on coloration then the same, but so is the degree of its expression. That is to say, it continues to show full heterozygous effect. It is interesting to contrast this result with that obtained when a gene has been introduced into a hybrid one of whose parents belongs to a species not known to possess it (see pp. 468-9).

The white females of *C. christina* Edw. are similarly dominant, but intermediate forms frequently occur which Gerould attributes to the action of modifying factors.¹ He has also pointed out that the dominant white females (*alba* Stgr.) of the European *C. myrmidone* Esp. are not infrequently free from the recessive lethal in nature. Near Graz about 22 per cent of the females are white, while they exceed the yellows in the mountains of Upper Styria. Although the European *C. croceus*

¹ It is possible that some confusion exists in regard to this species. Gerould (1923) remarks: "*Colias christina* Edw., of south-western British America and the north-western United States immediately east of the Rocky Mountains, presents the most bewildering variety of colours of any American stock of this highly variable genus." On the other hand, Seitz (1907) simply states that "the female is almost white..." and makes no mention of any yellow form. The only locality he gives is the Slave River (Alberta and Mackenzie).

Fourc. has been bred by many collectors, the control of the white female form (*helice* Hbn.) has not been so carefully studied as in *C. philodice*. It is, however, known to be dominant; for, though it always forms rather a small percentage of the population, captured specimens of *helice* have generally produced white and yellow females in about equal numbers. Its proportion in nature seems to vary considerably in different years. Thus in 1877 Nash (1929) found about one *helice* in every 200 specimens (males and females?), while in 1928 he caught about 133 females of which 17 were *helice*. It is curious that Heslop (1929) bred from a normal female and obtained 40 specimens of which only 3 were white, but he does not state how many of the remaining 37 were males. Chapman (1905) captured a *helice* female in France which must have paired with a heterozygous male; for the offspring consisted of 79 males, 52 *helice* and 19 yellow females. The presence of a recessive lethal, linked with the dominant colour gene, has not actually been demonstrated in this species. In the last example the proportions of the females are nearer 3 : 1 than 2 : 1, but they do not differ significantly from the latter ratio ($\chi^2 = 1.4$, with one degree of freedom, so that $P > 0.2$).

In all these *Colias* species the whitish females are subject to a good deal of variation in tint. The pale yellowish, creamy, or white forms so produced have sometimes received separate names. These are not worth quoting. Such minor fluctuations are probably genotypic (see p. 490); as already noticed, they seem to be more marked in *C. christina* than in the others which have been discussed.

When polymorphism is confined to one sex, it affects the female much more often than the male. All the instances so far discussed have been of this kind, as are most of the mimetic examples to be considered later. It is usually held that such sex-controlled inheritance is due to the operation of autosomal genes whose action is inhibited in the internal environment provided by one of the sexes, generally the male. Cockayne (1932 *a*) has however pointed out that the same result would be obtained if an autosomal gene could operate only in the presence of another gene carried by the Y-chromosome. It seems to me quite probable that this may indeed represent the situation in certain instances. Furthermore, such a system would explain why the female sex in the Lepidoptera, rather than the male, is so frequently that to which such genic action is restricted: at least it would do so if we could see any reason why the auxiliary factor in Y should more often be activating than inhibiting.

On the other hand, I do not myself regard this mechanism as having a wide general application in the group. The control of the ordinary accessory sexual characters, alike in male and female, shows us that the operation of genes must often be inhibited (or activated) by the internal environment provided by one of the sexes. To postulate additional activating genes in the Y-chromosome, specific for each sex-controlled factor, seems to favour the more complex of two possibilities, from which we may be deterred by the precept of William of Occam. At the time this theory was brought forward, it was of use in explaining the difficulty, presented by certain very rare exceptions, in which a female sex-controlled character has been manifested by a male; for this could result from crossing-over between X and Y.

In recent years, however, it has become apparent that factors interact with the gene-complex to produce the characters for which they are responsible. Recombinations may therefore alter the effects which particular genes have been selected to give: a condition well illustrated by occasional heterozygous manifestation in genes normally recessive. Transferences of sex-controlled characters to the opposite sex are explicable on the same general basis. Finally it is possible that the greater frequency of female than of male polymorphism has an ecological significance (Carpenter & Ford, 1933, pp. 121-3).

However, unisexual variation restricted to the male is not unknown in the Lepidoptera, and of this the moth *Parasemia plantaginis* L. provides an example. The males are normally yellow with black markings, and the females roughly resemble them, though the hindwings are sometimes marked with red (p. 477). In a variety of the male, known as *hospita* Schiff., the yellow is replaced by white. No corresponding form of the female exists. The species is to be found from sea-level to considerable altitudes, but the white variety is only known in certain mountainous localities, where it forms a small proportion of the male population. Near Kendal, in the English Lake District, Mr F. Littlewood has estimated this at about 2 per cent (Cockayne, 1932*a*). Littlewood secured two pairings with *hospita* but, unfortunately, he kept no exact records of his results. However, he certainly obtained specimens of this form among the F_1 offspring, and subsequently estimated that they had amounted to about 20 per cent of the males. Cockayne (1925*a*) captured a female at Rannoch (Perthshire) and bred 30 females and 30 males. Of the latter, 7 were *hospita*. He suggests that the form may be a dominant, but points out that the ratios depart rather widely from expectation on such a view. The deviation from equality is in fact significant ($\chi^2 = 8.5$).

However, this theory is supported by the results of Walther (1927), who captured a female of the var. *subalpina* in the Tyrol, which had paired with an unknown male. This produced an F_1 consisting of: males, 14 *hospita*, 11 *subalpina*, 7 *plantaginis*; and females, 1 *plantaginis*, 2 transitional to *subalpina*, 19 full *subalpina*. Of these, a male and female, both *subalpina*, were mated to produce the following F_2 generation: males, 7 *hospita*, 2 *plantaginis*, 6 *subalpina*, 5 *elegans* Rätz; females, 18 *subalpina*. *Hospita* is clearly behaving as a dominant here. Being rare, one only of the two original parents may be presumed a heterozygote, as the female parent of F_2 seems to have been. The lighter markings on the hindwings of *elegans* are white, and those of the *subalpina* males are yellow. The former may therefore be included in the *hospita* and the latter in the non-*hospita* totals. When this is done, the approach to equality in the males is satisfactory throughout.

The relation of *hospita* to the other forms is, however, far from clear. The lack of free assortment in F_1 suggests linkage. *Elegans*, appearing in F_2 only, is presumably a combination of *hospita* and *subalpina*. However, it differs from the latter not only in the absence of yellow pigment (as in *hospita*), but in a still greater extension of the black markings. It is curious that a *hospita*-like form with the normal markings of *subalpina* also exists (var. *borussia* Schaw.). Further study is certainly required to throw light on these points.

Several instances have been examined in which polymorphism affects both sexes. Bovey (1936) showed that the forms of *Zygaena ephialtes* in which the red or yellow pigment (p. 477) is restricted to the two basal spots on the forewings (*ephialtes* group) are recessive to those in which it is extended, appearing in the remaining forewing spots and on the hindwings also (*peucedani* group). The heterozygotes, however, are variable. The geometrid moth *Angerona prunaria* L. is orange in the male and pale yellow in the female, dusted with black spots in both sexes. A dark form, banded only with the normal coloration, ab. *corylaria* Thun. (*sordiana* Fues.), is much less common and is inherited as a single dominant¹ (Doncaster & Raynor, 1906). The evidence obtained by Raynor (1905) shows that the form of *Dysstroma* (*Cidaria*) *truncata* Hufn., with the discal area of the forewings yellow (var. *commantata* Fb.), is dominant to that in which it is white or brown. Perhaps 10–15 per cent of the species are of this yellow-marked variety. Doncaster (1907) pointed out that the breeding experiments conducted by Prout (1906) on *Xanthorhoe ferrugata* Cl. clearly demonstrate that the purple-banded form is dominant to the black-banded, a conclusion which the latter author had failed to reach. This purple form is generally much the less common of the two, but it is said to be the more frequent in the Isle of Man. The back-cross purple \times black-banded produced 171 black-, 147 purple-banded, and 4 intermediates. The latter were probably due to a failure of dominance. Selection experiments conducted on the heterozygotes might indicate that this can easily be modified. It may be added that the nomenclature of this species is in some confusion. It has long been known as *unidentaria* Haw., while *ferrugata* Cl., which must be adopted for it (Seitz, 1912), has been used for the allied *X. spadicearia* Schiff.

The polymorphism of *Danaus chrysippus* L. has not been investigated genetically, but it would be of interest from this point of view. The butterfly is extremely common throughout the warmer parts of the Old World. The form *dorippus* Klug., which lacks the black and white subapical bar on the forewings, is especially characteristic of certain desert regions. It is known in Arabia and in parts of the Punjab. In East Africa it flies with, but outnumbers, the normal form in some areas; but it has only been recorded once or twice in South Africa, and never on the west coast. We may expect that this dimorphism, which affects both sexes, is of a simple kind. Lamborn (1924) captured a female *dorippus* which produced two *chrysippus* offspring. Van Someren (1924, 1925) also obtained a family from a *dorippus* female, which consisted of 1 *chrysippus* and 5 *dorippus*. It is not improbable that the latter will prove to be a heterozygous form. The species acts as a model for many others, but its own polymorphism cannot be regarded as mimetic.

In none of the foregoing examples of polymorphism affecting both sexes has it been established what check prevents the spread of the more favoured form. The homozygous dominants may well be associated with some lethal condition, but this has not actually been proved.

¹ There is some evidence that dominance is here incomplete, for the heterozygotes seem to be speckled. Both in this and in the other instances of dimorphism discussed, further data may reveal a deficiency of the homozygous dominant class.

(2) *Mimetic polymorphism*

I propose to deal only briefly with the genetics of mimetic polymorphism. This is an important subject, but I have discussed it elsewhere (Carpenter & Ford, 1933; Ford, 1936). I shall therefore confine the present account to a short survey of the factors so far analysed and a discussion of a few of the problems which they raise.

The males of the African butterfly *Hypolimnas misippus* L. are non-mimetic and characteristic of their genus. The females are dimorphic and utterly unlike them. One form, *misippus*, mimics the normal *Danaus chrysippus*; the other, *inaria* Cram., copies the *dorippus* form of its model. These are controlled by a single factor pair, *inaria* being recessive (Rogers, 1911, 1912).

A situation which may be genetically very similar is found in the North American *Papilio glaucus* L. The males and females normally resemble one another, being yellow with black markings. This form of female (called *turnus* L.) is the only one found in the northern part of the species' range. However, in the south and south-west, from New England to Florida, a second female form (*glaucus*) also occurs. It is blackish, and mimics *Papilio philenor* L. (Poulton, 1908). Unfortunately the genetics of this butterfly have not received detailed study. Edwards (1884) bred from it and obtained a few small broods, of which he gave a somewhat confused account. He says that *glaucus* females produce only their own form, or else a majority of it and a few *turnus*. Cockayne (1932*a*) plausibly suggests that the latter is a simple recessive. A few intermediates have been recorded by collectors, and these are probably due to a failure in dominance.

Both sexes of the African *Hypolimnas dubius* Beauv. are dimorphic and excellent mimics. On the west coast one form, *dubius*, copies *Amauris psytalea damocles* Staud., while the model of the other, *anthedon* Doubl., is *Amauris niavius niavius* L. The difference between them is uni-factorial, *dubius* being the dominant (Lamborn, 1912). On the east coast these two forms have been selected to copy other models, for those utilized on the west are not available. The black and white *dubius* is converted into *mima* Trim., with a yellow basal area to the hindwings, in mimicry of *Amauris albimaculata* Butl. and *A. echeria* Stoll. *Anthedon*, on the other hand, retains the same species as a model, but follows its geographical race (*A. niavius dominicanus* Trim.) by extending the white area on the hindwings, and is known as *wahlbergi* Wallengr. These eastern forms have been bred from captured females on a number of occasions, giving either offspring all similar to their parent or segregation in a 1:1 ratio. Platt (1914), however, found two *wahlbergi* in copula, from which he raised 104 *mima* and 94 *wahlbergi*. This seems to suggest that the latter is the dominant, though the corresponding form on the west coast is the recessive. I have, however, given reasons for thinking that in this instance the female parent had mated twice (Carpenter & Ford, 1933, pp. 117-18). It is in reality probable that *mima* is the dominant in the east, as is its corresponding form *dubius* in the west.

When mimetic polymorphism involves more than two alternative forms, a genetic situation of much interest arises. The best known study of such a condition

is that of Fryer (1913) on *Papilio polytes* L. This is an Oriental species in which the males are monomorphic and the females polymorphic. One female form (*cyrus* Fabr.) resembles the male, another (*polytes*) is mimetic of *P. aristolochiae* Fabr., while a third (*romulus* Cram.) mimics *P. hector* L. Fryer showed that a sex-controlled dominant factor (**A**) converts *cyrus* into *polytes*, and that a second sex-controlled dominant (**B**) converts *polytes* into *romulus*. However **B** produces no detectable effect except in the presence of **A**. Thus all individuals possessing *aa* are *cyrus*, *polytes* must have at least one **A** combined with *bb*, while the presence of at least one dominant factor of each pair produces *romulus*. Both factor pairs are autosomal.

The genetics of *P. memnon* L. have been investigated by Jacobson (1909) and analysed by de Meijere (1910). This author studied the species in Java, where a number of female forms occur, but he confined his work to three of them. The commonest of these, *laomedon* Cr., somewhat resembles the monomorphic male. A single dominant factor (**A**) converts it into a second non-mimetic form *isarcha* Seitz.¹ Neither the male nor these two female forms possess tails. However, a second dominant gene (**B**), working in conjunction with the first, produces the tailed mimic *achates* Sulzer, which copies *P. coon* F. The factor **B** has no effect in conjunction with *aa*, so that the control is quite similar to that just described in *P. polytes*. Indeed it will be seen that, genetically, *laomedon*, *isarcha*, and *achates* respectively resemble the *cyrus*, *polytes* and *romulus* forms of that species. The polymorphism of *P. memnon* is, however, the more striking, since a single factor governs not only colour and pattern, but also shape; for it determines whether or not the females shall develop tails.

The various forms of *P. dardanus* Brown provide by far the most complex example of mimetic polymorphism. I have, however, recently compiled a full account of them, in which I have brought together the results of many breeding experiments on this species and discussed its genetics in detail (Ford, 1936). It is therefore unnecessary to give more than the briefest summary of the subject here.

P. dardanus is found throughout the Ethiopian region, but not elsewhere. It forms eight subspecies, which merge into one another and interbreed at their boundaries (except for the Madagascan race *meriones* Felder). The males are non-mimetic, tailed, and monomorphic. The females, however, comprise an extraordinary assemblage of diverse polymorphic forms, many of which are subject to modification and minor variation. In a few districts they are tailed, non-mimetic, and male-like. Generally, however, they are mimetic, utterly different from the males and, like their models, are tailless; but a few tailed mimics are known. There are also several female forms which are non-mimetic although unlike the males. These are very variable, as also are the mimetic forms themselves in the mountainous districts of Tanganyika territory where their models are rare. Normally, however, these mimics are extremely constant both in colour and marking. We have here a valuable example of the fact that selection tends to produce stability.

On the west coast of Africa nearly all the females are of the form *hippocoön* Fab.,

¹ Jacobson calls this *agenor* L. But Cockayne (1932 a) points out that this form does not occur in Java, and that he probably worked with *isarcha*. Seitz (1908 a) considers that *agenor* = *javanus* Haase.

mimicking *Amauris niavius niavius*. A single factor converts it into the rare non-mimetic variety *dionysos* Doubl., which is a heterozygote; its homozygous effect is not known. In the east, the non-mimetic *natalica* le Cerf is probably a heterozygous modification of *hippocoonides* Haase (the form of *hippocoon* mimicking *A. niavius dominicanus*). *Cenea* Stoll (a mimic of *A. echeria* and *A. albimaculata*) is dominant both to *trophonius* Doubl. and to *hippocoonides*. A single factor converts the latter form, but not *cenea*, into *trophonius* (which copies *Danaida chrysippus*). The genetic relation of these three forms is therefore similar to that of the three forms of *Papilio polytes* and *P. memnon* already discussed. It is, however, uncertain whether *trophonius* is recessive or dominant to *hippocoonides*. Its west coast representative *trophonissa* Auriv. is probably converted into *niobe* Auriv., a mimic of *Bematistes* (*Planema*) *tellus* Auriv., by a single "modifying factor". The "*proto-salaami*" form is dominant to *hippocoonides*, and *poultoni* Ford is a genotypic modification of it.

So striking are the differences between the forms of polymorphic mimics, that it has even been felt difficult to attribute their control to the "normal" action of a single factor pair. Thus, after quoting several instances of female mimicry in which single factors produce profound changes, Goldschmidt (1923, p. 150) remarks: "we are here concerned not merely with a single factorial difference but a reaction of this with the hormones of female differentiation. Now it is exactly this sex hormone action that is able to direct the same substratum in two altogether different directions". The occurrence of equally extreme uni-factorial polymorphism affecting both sexes of a mimic, as in *Hypolimnias dubius*, appears to have escaped his attention.

Endless controversy has taken place over the evolution of mimetic patterns. In particular, the genetic control of mimetic polymorphism presents problems of exceptional difficulty and importance. On the one hand, the resemblance of each form to its particular model may involve the most striking adaptations *in a number of distinct characters*: such as colour, marking, shape, and even habit. On the other, the control of two such highly adapted and widely different forms may be dependent upon a single genetic factor. The allelomorphic difference thus involved must have originated by spontaneous mutation. Yet the numerous instances of this kind make it impossible to believe that selection has had to wait upon the fortuitous occurrence in each of a mutation having the varied effects required. This would be an almost impossible conclusion even if a single species only had been involved.

Punnett (1915) has endeavoured to explain these facts by assuming that parallel mutations have occurred in model and mimic; the same gene thus evoking the same set of characters. This view has lately been revived (Watkins, 1935), but a close study of the insects themselves makes it quite untenable. Indeed one of the most consistent features of mimicry is its complete superficiality. Though the patterns of model and mimic may be extremely alike in their general appearance, they are nearly always very distinct in their detailed marking, the similar colours seem often to be produced by different pigments or in a different manner, and indeed the

corresponding effects are generally attained by surprisingly diverse means (Fisher, 1930, pp. 155-8).¹

When we find apparent similarities between models and mimics constantly dependent upon devices of a fundamentally different kind, it is impossible to regard them as due to parallel mutation. It may also be mentioned that such a view necessitates a corollary which I believe few workers would be willing to accept: that the differences (e.g. in pattern and colour) between dissimilar, and often not closely allied, species are themselves uni-factorial if copied by a mimic exhibiting uni-factorial polymorphism.

To the writer it appears that the only evolutionary theory which is in harmony with the apparently conflicting facts is the one originally suggested by Fisher (1927). He pointed out that the effects of a gene may be modified by selection operating on the total gene complex. Consequently, when any mutation chances to give a remote resemblance to a more protected species, from which some advantage, however slight, may accrue, the deception will constantly be improved by selection. This, working upon genotypic variability, will result in a gradual change in the *effects* of the gene concerned, which may come to be very different from those which it had at first. But the gene itself is unchanged, and remains as a switch turning on one or another set of characters subject to genotypic variability and, consequently, susceptible of selection. Thus, though the forms of a species may be controlled by a single factor difference, which must have originated suddenly by mutation, we are none the less entitled to regard them as the product of slow evolution.

I have shown elsewhere that intermediates can be found between alternative mimetic forms controlled by a single gene, and that such aberrations are genotypic (Carpenter & Ford, 1933). Their existence is by no means in accord with the explanation of mimicry on the theory of parallel mutation. They do, however, represent precisely the type of variation which we are here postulating as providing a basis upon which selection may bring about gradual evolutionary change.

IX. SUMMARY

1. In this article I have discussed problems of special importance in the genetics of the Lepidoptera.
2. It is restricted to a consideration of the effects of individual genes; but those controlling sex, and the genetics of *Bombyx mori* as a whole, are omitted for the reasons given on pp. 461-3.
3. An index is provided, which will make it easy to ascertain what genes are known in the various species.

¹ To take an example from one of the best known instances of mimicry: the red pigment on *Papilio aristolochiae* and *P. hector* is changed to orange or bright yellow by hydrochloric acid, and can be reconverted to its original colour by ammonia. Thus it seems to be of the same type as that discussed in Section VI. But the red coloration on the two forms of *P. polytes* which mimic them behaves quite differently. Hydrochloric acid affects this to a very small extent only, and in quite another way, producing a slightly darker and more purple tone without any trace of yellow. Clearly parallel variation is not at work here: the resemblance in reality depends upon pigments of different chemical constitution.

4. The nomenclature has been brought up to date, and the author of each name is given the first time it is used. Discarded names are also included if these have become very well known.

5. It has been found on a number of occasions that crosses involving contrasted characters lead to the production of intermediates, with no clear-cut segregation in subsequent generations. There is no ground for regarding this type of inheritance as non-Mendelian. We have, furthermore, a decisive test in such circumstances. If the F_2 generation is more variable than the F_1 , a particulate, not a blending, mechanism must be involved.

6. Cytoplasmic inheritance has been detected in a few instances in the Lepidoptera. It is, however, unlikely to play anything but a most subordinate part in their heredity.

7. Larval and imaginal characters are usually controlled independently, even when changes of a similar kind are involved. This is to be expected on evolutionary grounds, and presents no genetic difficulties.

8. A species cross is discussed which provides strong support for the evolution of dominance by selection acting on the gene complex.

9. A short summary is given of the genetics of larval characters, and a number of physiological and other problems raised by it are considered.

10. The fact that very few instances of sex-linkage have been detected in the Lepidoptera is probably due to the large number and small size of the chromosomes in this order. The similar lack of information on autosomal linkage will be due, in addition, to the infrequency with which any dihybrid crosses have been studied. It is suggested that a multiple allelomorph may have been obtained at the *dohrnii* locus of *Abraxas grossulariata*.

11. An account is given of the genetic control of characters separating geographical races.

12. Special attention is drawn to two classes of pigments which provide fruitful material for combined genetic and chemical investigation.

(a) White and bright yellow pigments are often developed in the Pieridae. In the species so far studied chemically, they are due to the allied compounds leucopterin and xanthopterin respectively: both are related to uric acid. A very rare bright yellow form of the white *Pieris napi* is certainly inherited on a simple basis. It is highly probable, but not yet proved, that the genes concerned determine the alternative production of these two substances. They may also be developed in the white and yellow forms of *Abraxas grossulariata*, which are under a uni-factorial control.

(b) A characteristic pinkish red pigment is formed in a number of distinct groups of moths. It is changed to bright yellow by strong mineral acids (e.g. hydrochloric). But it can then be reconverted to red by an alkali (ammonia). Furthermore, in nearly all these species, genotypic varieties occur in which the red colour is replaced by bright yellow in the living insect. This is, however, unaffected by ammonia. It is probable that these two pigments (red and yellow) are closely allied, and easily convertible the one into the other. A survey is given of the genetic control by which this seems to be achieved.

13. The existence of melanism in non-industrial and in industrial areas is treated separately. The genetic mechanisms encountered within each type are discussed, together with a number of relevant problems.

14. Special attention is drawn to the dominance relations of certain melanic forms.

15. The various theories accounting for the recent spread of melanism in industrial areas are considered. It is concluded that none of them are entirely satisfactory, and a new view is advanced. It is held that the chief agent in this change is selection operating in favour of characters other than colour, produced by genes normally present as rarities. When, as already proved in some instances, these combine melanin production with a physiological advantage, it is suggested that they cannot spread save in industrial areas, owing to counter-selection against black coloration. This theory is confirmed by the existence of melanic varieties hardier than the normal form which, however, they do not supplant in unpolluted country.

16. A review is given of the various mechanisms by which non-mimetic and mimetic polymorphism is maintained.

17. The different forms of a mimic may each copy their respective models by striking modifications in a number of distinct characters. Yet the control of two such highly adapted forms is often dependent upon a single genetic factor. This must have arisen spontaneously by mutation but, in view of the numerous instances of such mimicry, it is impossible to believe that selection has had to wait for the fortuitous occurrence of mutations each evoking the required train of effects.

18. The complete superficiality of the resemblances between mimics and models invalidates the suggestion that they are due to parallel mutation.

19. It appears that the only evolutionary theory in harmony with the facts is the view that the *effects* of genes chancing to give some slight superficial resemblance of mimic to model have been modified by selection operating on the gene complex: such genes themselves remaining unaltered, to act as a switch in maintaining the alternative forms.

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XI. INDEX

This is intended as a guide to the genetic factors mentioned in this article, not as a general index. Many species introduced incidentally are therefore omitted. Furthermore, the references to those listed here give only the pages on which will be found an account of the genes occurring in them. Such species may appear in general discussions elsewhere in the paper, but they are not indexed at such places.

The generic and specific names adopted are throughout in accord with the most recent advances in nomenclature (p. 463). However, a few of those which have become obsolete are so well known that it has been thought a convenience to include them also. The author of each name may be found by reference to the first page on which it occurs.

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ADDENDA

While this article was going through the press, Hawkins¹ published an account of his interesting breeding experiments on *Ptychopoda aversata* L. This is a dimorphic species, having an abundant unbanded form, *remutata* L. (= *spoliata* Stgr.), and a less common banded one, *aversata*. The latter may be of either sex, and apparently exists throughout the range of the species, occupying, at a rough estimate, perhaps 5 per cent of the population. Hawkins has shown that this dimorphism is controlled by a single factor pair, the plain form being homozygous and the banded heterozygous. Actually, he describes the latter as a dominant but, as he obtained no homozygous banded specimens, it is not in reality known if they are identical with the heterozygotes.

A further point remains to be studied. Hawkins himself remarks that there is no reason to suspect lethal factors in this species. As he was dealing only with the commoner homozygous type and the heterozygotes, this view is justified as far as his own experiments were concerned. On the other hand, as the situation is one of balanced polymorphism, the existence of differential viability affecting adversely the homozygous banded individuals is quite probable (p. 489). Thus further work may well reveal that the homozygous "dominant" class is disproportionately rare or even absent. Should it prove to be so, it would be interesting to determine, if possible, what counterbalancing advantage preserves the banded form in the face of reduced homozygous viability.

¹ Hawkins, C. N. (1937). *Entomologist*, 70, 25-7.

Further breeding experiments on *Hemerophila abruptaria* Thnbg. have recently been conducted by Brett,¹ who finds that he can distinguish two melanic forms: the dark chocolate var. *fuscata* Tutt, and one of a paler shade. The latter has occurred in small numbers in his broods, insufficient to determine its genetic status, but he notes that females are much commoner than males. True *fuscata*, on the other hand, seems to be distributed equally between the sexes.

It has already been mentioned (p. 483) that Onslow (1921 c) regards melanism in this species as due to a simple dominant, and Brett's results on the whole confirm this view. However, he encountered certain irregularities. Thus he obtained 1 melanic and 37 normals from a pairing between two normal specimens. Onslow also obtained 1 melanic out of 40 individuals in similar circumstances. This he attributed to an experimental error, but it now seems more probable that some genetic complication is involved. However, as Onslow did not separate the light and dark melanics, his data cannot be used to elucidate the point.

Brett also throws doubt on Onslow's statement that the melanics are hardier than the normals (p. 487), attributing the excess of the former class in some of Onslow's broods to irregularities due to the unsuspected existence of two forms. It may be noticed, however, that this occurs in the families subject to disease, in which the effect of differential mortality might be the more pronounced, and appears significant. (His numbers were, 37 melanic : 19 normals, being totals of his back-cross broods in 1920. The departure from equality is here measured by $\chi^2 = 5.8$ with one degree of freedom, so that $P < 0.02$.) In view of the fact that the superior viability of the melanics has been established in a number of species in which melanism is dominant (p. 487), the same condition may reasonably be expected in *H. abruptaria*. Furthermore, Brett's own segregating families depart from expectation in favour of the melanics. In the F_2 broods the deviation from a 3 : 1 ratio is not significant, though it might become so with larger numbers (103 melanics : 28 normals; so that $\chi^2 = 0.92$, and $P > 0.3$). But the departure of his back-cross from equality certainly suggests significance (23 : 9, giving $\chi^2 = 6.1$). However, further data must accumulate before these results can be interpreted with certainty.

It was pointed out on p. 478 that black specimens of *Papilio machaon* (ab. *nigra* Reutti) have been captured on a few occasions. By an oversight, I had omitted to mention the results of breeding this form.²

A stock, originating from Ranworth larvae, produced large numbers of typical specimens for two generations. However, among many normal *machaon* comprising the third generation were at least seven ab. *nigra*. One of these, a female, was paired with a typical male from the same stock. It produced 13 normal offspring and 6 melanics (3 ♂, 3 ♀).

As the authors point out, the black form cannot be a simple dominant, but it may be a recessive. On the other hand, they remark that the numbers (13 : 6) are not very close to the expected 1 : 1 ratio, though the black specimens may be the more delicate. They therefore suggest, alternatively, that the melanism may be controlled by the interaction of two genes, each without effect by itself, and they show that segregation in a 10 : 6 ratio could be obtained on this basis. However, the observed numbers do not, in fact, differ significantly from equality ($\chi^2 = 2.57$ so that, with one degree of freedom, $P > 0.1$). Consequently we may provisionally regard the black form of this species as recessive and uni-factorial.

¹ Brett, G. A. (1935-6). *Trans. S. Lond. ent. nat. Hist. Soc.* pp. 84-92.

² Cockayne, E. A. and Newman, L. W. (1931). *Proc. ent. Soc. Lond.* 6, 95-6.

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